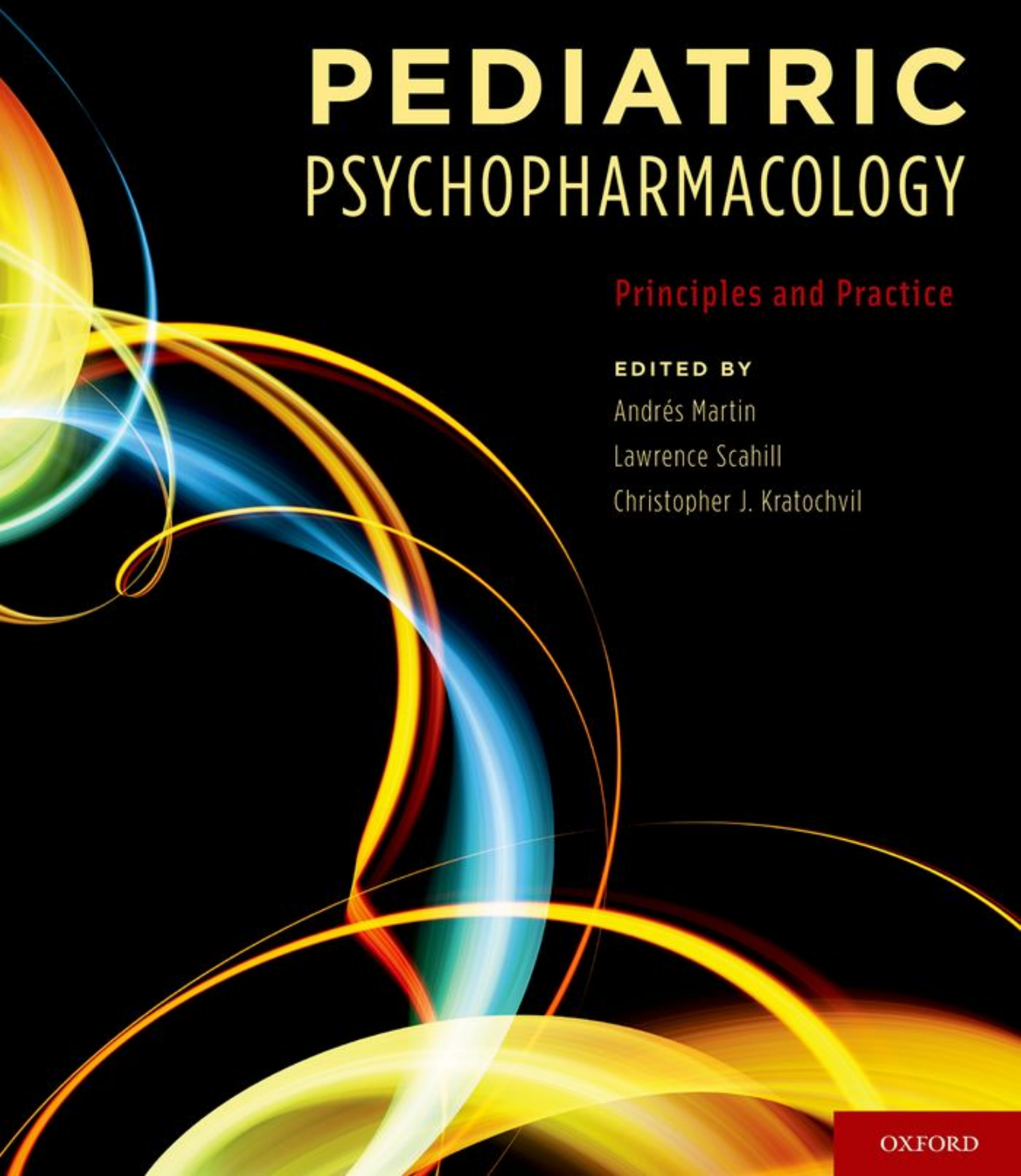
SECOND EDITION

PEDIATRIC PSYCHOPHARMACOLOGY

Principles and Practice

EDITED BY
Andrés Martin
Lawrence Scahill
Christopher J. Kratochvil



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Pediatric Psychopharmacology

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Principles and Practice

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IN MEMORIAM

Donald J. Cohen, MD
September 5, 1940 – October 2, 2001

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Foreword to the First Edition

It is still early in our understanding of the pathogenesis and treatment of psychiatric disorders—the brain, after all, is the most complex object of study in the history of science. Knowledge about the pathogenesis and treatment of pediatric psychiatric disorders has been even harder-won than knowledge about adults because superimposed upon the inherent difficulties of understanding brain and behavior are the added challenges imposed by development. Pediatric psychopharmacology has faced additional hurdles. It has special ethical challenges in the conduct of its clinical research, including clinical trials. Moreover, pediatric psychopharmacology emerged against the backdrop of entrenched prescientific approaches to childhood psychiatric disorders that often had strong biases against drug treatment for children—biases based entirely in untested theory. Despite these hurdles, the last decade has seen pediatric psychopharmacology emerge as a discipline with a growing knowledge base and an increasingly well-defined set of research goals. The progress in pediatric psychopharmacology evidenced by this volume is particularly important for the sake of the children that it will benefit. Psychiatric disorders that occur in childhood not only cause suffering, disability, and family distress, but may also cast a life-long shadow. A child with an untreated mood or anxiety disorder, for example, or untreated attention-deficit hyperactivity disorder may have difficulties learning in school and problems developing healthy family and peer relationships. Over time, such children exhibit elevated risks of developing secondary psychiatric disorders, including substance use disorders, and of school failure and legal difficulties. Early and appropriate diagnosis and treatment can avert such downward spirals.

This volume brings together our best current understandings of relevant neurobiology, pharmacology, and treatment for a broad range of childhood mental disorders. As such, it is both significant and useful. I sincerely hope, however, that the authors of this volume are dedicated to this project for the long haul. That is because, as significant as the progress of the last decade has been, we are just at the beginnings of what we must achieve both in basic science, clinical investigation, and treatment recommendations. Emerging approaches to brain and behavior derived from sources as diverse as the genome project and cognitive neuroscience are creating new opportunities for research in pediatric psychopharmacology, but their wise use will require hard work, new ideas, and ingenuity. For example, genetic approaches to pathogenesis and treatment development, while very promising, have also proven extremely difficult. Perhaps it should have come as no surprise that the genetic contribution to risk of abnormalities in cognition, emotion, and behavioral control are based not on single genetic mutations but on the complex interaction of multiple genetic variants and nongenetic factors. Similarly, deep understandings of human brain development and its relation to behavioral maturation and disease risk will not be easily achieved. It is only in recent years that a basic platform for such understandings, atlases of human brain anatomy as it changes over the course of development, have begun to emerge. Other critically important areas of science are only just beginning to coalesce. One such area that should have an impact on pediatric psychopharmacology is social neuroscience, the attempt to show how the brain supports social behavior, including interpersonal interactions, and to understand how social interactions

shapes the brain. Even for existing medications, much remains to be done in the arena of treatment studies. Well-designed and larger-scale clinical trials for such common illnesses of children and adolescents as depression and anxiety disorders have either been only recently completed or are still under way. Clinical trials in young children are much in need, but raise daunting ethical questions.

With full recognition that we still have far to travel, the appearance of this volume documents the fact that pediatric psychopharmacology has reached its own important developmental milestone. What is contained in this volume and the implied promise for the future will be of enormous benefit to children and families.

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Preface to the Second Edition

Eight years have passed since the publication of *Pediatric Psychopharmacology: Principles and Practice*. The near-decade has been a time of major developments and progress in the field, yet proved laden with serious challenges as well. This second edition is a comprehensive scholarly source on the use of psychotropic medications in children and adolescents and captures all facets of a rapidly evolving and maturing discipline. We begin by highlighting some of the many advances and the excitement that have accrued between the two editions of the book.

One of the most salient developments during this time has been the completion of a series of federally funded multisite clinical trials, as described in detail in the book's third part. Under a veritable alphabet soup of abbreviations, these systematic research efforts have provided much-needed guidance on how to approach the treatment of children with depression (TADS), treatment-resistant depression (TORDIA), obsessive-compulsive disorder (POTS), autism (RUPP), attention deficit in preschoolers (PATS), and early onset psychosis (TEOSS), to name but a few examples. These multisite efforts have built on the model first implemented successfully with the MTA study, to date the largest clinical trial in pediatric psychopharmacology, and an ongoing source of clinically relevant information nearly two decades after its inception. In addition to epitomizing fruitful collaborations across institutions and between government funding agencies and academic sites, these studies have moved the field beyond the simple comparison of an active treatment to a placebo—they have also offered information on how to sequence and integrate psychiatric medications with psychotherapy and other non-biological approaches.

The advances in basic human neurobiology in general, and in genetics and neuroimaging in particular, are in good evidence in the first part of this new edition. A more refined understanding of the fundamental mechanisms in brain circuitry underlying attention systems is one particularly gratifying example. In this area, science has advanced our understanding to the point where we can start "connecting the dots" between how genes, proteins, circuits, and effective treatments are interrelated. As a result, we have been able to refine the application of interventions at our disposal. In other areas, such as in Fragile X syndrome, fundamental neurobiology has opened potentially new avenues for intervention, offering the promise of entirely new compounds that may one day revolutionize how we treat, or indeed prevent, mental illness. Although many of these leads are still in an early phase and have not yet borne the fruit of their clinical promise, they do invite us to reconsider the great potential of this still young field.

The second part of the book, focused on the specific pharmacological agents available today, has perhaps been the slowest one to advance. This may be a simple reflection of the fact that the fruits of neurobiological research are slow to mature, particularly when it comes to advancing new compounds across the pipeline of pharmaceutical development. Indeed, one can argue that there has been no entirely new compound introduced in pediatric psychopharmacology since the first edition of this book: even though atomoxetine had not made its appearance in it, the agent had been in existence and unsuccessfully examined for the indication of depression several decades before. Disappointing as may be the fact that no mechanistically new

medications have emerged in the past decade, there have been notable advances in the introduction of more "user-friendly"compounds—medications with pharmacokinetic properties that permit less intrusive dosing regimens and result in fewer adverse drug interactions. There has been progress no doubt, but we believe that the best is yet to come, with truly revolutionary and "game changing" novel agents to be developed in the years ahead. In order for this enormous potential to be realized, strong partnerships across academic, government, regulatory, and pharmaceutical sectors will be necessary.

A precondition toward such fruitful collaborations, as well as a byproduct of them, will be a regaining of the public's trust. For one of the more challenging aspects facing pediatric psychopharmacology since the first edition of this volume has been questions raised about potential conflicts of interest between investigators and the pharmaceutical industry. Independent of the eventual outcome of those often-charged discussions, it is evident that remarkable changes have ensued over the past decade. For example, the disclosure by authors of their potential conflicts of interest was not the norm in many academic periodicals, and certainly not in edited textbooks such as this volume. Such statements are not only de rigueur by now (and included at the end of this book), but in fact an entire new chapter regarding conflict of interest has been added to the fourth part of this revised edition. It is clear that additional thought and discussion of these issues are warranted.

Important clinical questions regarding the potential for SSRIs to induce suicidal ideation that emerged shortly after the publication of the first edition led not only to the eventual blackbox warning now affixed to all antidepressants, but also contributed importantly to the establishment of clinical trial registries that are now the law of the land across the entirety of medicine—well beyond the narrow confines of pediatric psychopharmacology. The explicit discussion and management of potential conflicts of interest, and the institution of clinical trial registries are two instances that are obviously not the exclusive domain of pediatric psychopharmacology, but that have certainly left an important imprint on the field.

In an editorial published in the *Journal of the American Medical Association* in April, 2010, NIMH Director Thomas Insel suggested that rather than being passive bystanders at the crossroads of such exchanges, we stand much to gain by taking an active role in crafting relationships that should be as sound ethically as they are scientifically:

> The focus on financial conflicts of interest in psychiatry is an opportunity to take the lead in setting new standards for interactions between all medical disciplines and industry. Academic leaders, professional societies, and patient advocacy groups could turn the tables of public trust by developing a culture of transparency for psychiatry's collaborations with industry, including the clear separation of academic-clinical missions from industry marketing.

Each reader can decide whether the changes across editions have been too few or too many; whether the field has changed too gradually or moved ahead at too rapid a pace. Regardless of that subjective assessment, our goals and our commitment have remained steadfast. We have sought to put together a volume that is scholarly, comprehensive, and integrative, and to capture the most recent and cutting-edge information, yet remain grounded in the principles of good clinical care that have informed our own practice and that of our predecessors.

Three of those predecessors deserve specific mention. First, the late Donald J. Cohen, to whom this volume is once again lovingly dedicated, set a standard of clinical and scholarly preeminence that we seek to emulate to this day. As in the first edition, we have interspersed some of his writings and enduring thoughts between the four parts of the book. Second, Dennis S. Charney, Dean of the Mount Sinai School of Medicine, who had the initial idea for *Pediatric Psychopharmacology* and was its champion and rainmaker when still at Yale back in 2003. Finally, our dear colleague James F. Leckman, an indispensable partner in the first edition, and who

has remained a committed contributor in this second one. We are grateful to Dennis and to Jim for their original involvement with the book, and for their supportive and gracious passing of the baton. Neither edition could have come to be without their unique energy and vision.

In this new edition we have moved from an entirely Northeastern editorial quartet to welcome a Great Plains colleague into our fold. We are delighted by this new partnership, grateful for the contributions of our many authors, and eager to have the extensive knowledge contained in these pages reach out and help lessen the burden on psychiatrically ill children everywhere.

A.M., NEW HAVEN, CONNECTICUT
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Preface to the First Edition

The student who has the capacity to be parented—to appreciate the nurturance and to show this through his own growth—will develop the ability to parent: to be loyal to the values of his teachers by conveying them to his own students.

Donald Cohen (1986)[1]

If we take eternity to mean not infinite temporal duration but timelessness, then eternal life belongs to those who live in the present. Our life has no end in just the way in which our visual field has no limits.

Ludwig Wittgenstein (1922)[2]

Pediatric Psychopharmacology: Principles and Practice (PPPP) first became a concrete idea in the fall of 1999, shortly after the publication of the first edition of *Neurobiology of Mental Illness* (NMI). Under the lead editorship of Dennis Charney (together with Eric Nestler and Steven Bunney, when all three were still at Yale), NMI set a new standard for scholarship in the rapidly evolving field of biological psychiatry, and provided a powerful impetus and central model for this book. Like many others, we thought then that a critical mass of knowledge and evidence base had accrued in the field of pediatric psychopharmacology, such that the time was ripe for organizing a similarly comprehensive volume that was entirely devoted to the topic. Developing that original idea into the specific project that it has become today has been a challenging task involving difficult choices. Among the many decisions made, however, the easiest and least disputed of all proved in the end to be the most daunting. We agreed from the outset that Donald Cohen would be the optimal person to write the volume's introduction, as we could think of no one better able to contextualize its scope within the broader domain of child and adolescent psychiatry.

Things turned out very differently. Donald J. Cohen, MD, Sterling Professor of Child Psychiatry, Psychology and Pediatrics, and Director of the Yale Child Study Center since 1983, died at the age of 61 on October 2, 2001. As our friend, mentor, and colleague, Donald followed with interest and excitement the progress of the book, but in the end, his untimely death did not permit him to write what he had gladly agreed upon. And where there was to be his introduction now stand his photograph and a memorial note instead. Even if our loss is recent as we write these lines, this volume has proved to be an active way of mobilizing our grief inasmuch as it has become an effective vehicle to carry his vision forward. It has, in fact, made Donald present in our lives in unsuspected ways.

Donald Cohen did not see himself as a psychopharmacologist, much as he did not see himself as a therapist, a psychoanalyst, a researcher, or a policy-maker. Not only did he find fault with such traditional demarcations as applied to himself: he saw them more broadly as shortsighted truncations and pigeonholings of our field and

1 Cohen, D.J. (1986), Research in child psychiatry: Lines of personal, institutional and Career development. In: *Clinical Research Careers in Child Psychiatry*. H.A. Pincus and H. Pardes (eds.). Washington, D.C.: American Psychiatric Association, p. 74.

2 Wittgenstein, L. (1922), *Tractatus Logico-Philosophicus*, 6.4311.

our professional selves. A master humorist, with tongue in cheek he boasted about having the very same prescription pad that he had received upon graduating from medical school. Donald was the ultimate advocate for children and for child psychiatry, a specialty he saw at the core of pediatrics. He felt an enormous sense of pride on seeing the growth of the field during the decades of his active work, and remained the perennial optimist to the end, hopeful that its major breakthroughs and most effective therapies were near at hand.

As we completed this project, it became clear that Donald's vision was woven into the fabric of the entire volume. For example, we propose an integrated model that places psychopharmacology as but one (albeit a powerful one) among the many tools available for the treatment of psychiatrically ill children and adolescents. Much as he would, we advocate for thoughtfulness and restraint in the prescription of psychotropic drugs, for the judicious use of diagnostic labels, and for the conceptualization of childhood psychiatric disorders within a developmental framework. Rather than a pathway toward the diagnostic and therapeutic minimization of the psychiatrically ill child, we see (and practice on a daily basis) psychopharmacology as another avenue toward the deeper understanding and more effective treatment of affected youngsters. These overarching tenets are at the core of the volume. Even while bringing together a large body of knowledge and serving as a benchmark for the advances of the basic and clinical sciences that underlie psychopharmacology, this book is in some ways more than just dedicated to Donald Cohen: it is largely imbued with his vision for this novel and promising discipline. To highlight this fact, we have started this preface and each of the section introductions that follow with citations taken from his extensive body of writings.

The volume is divided into four major parts. The first, *Developmental Bases of Pediatric Psychopharmacology*, provides a foundation of neurobiology upon which the rest of the volume builds. The molecular and genetic mechanisms that underlie brain development and drug action are presented as fundamental building blocks for a larger subsection devoted to discrete diagnostic entities. That subsection, *Developmental Psychopathology*, expands on one of Donald's central views concerning the interplay between normal developmental trajectories, their disrupted course under disease states, and the individual's adaptation to such dysregulation, with particular attention to psychotropic drug effects in this instance. This part benefits richly from the genetic and brain imaging findings of the "Decade of the Brain," and as such reflects the increasing depth in our understanding of the workings of the brain under normal and pathological states.

The second part, *Psychotropic Agents*, reviews the current state of knowledge of the various drug classes routinely used in child psychiatry. It pays special attention to age-specific differences relevant to medications actions, including variations in response or side effect profiles, as well as regulatory issues pertaining to children. Chapters are included that address other somatic interventions, such as complementary and alternative medicine approaches, as well as electroconvulsive and related treatments. The chapter devoted to the α agonists is, among other things, a fitting tribute to the man who first proposed the use of clonidine for the treatment of a child psychiatric condition in the 1970s.

The third and longest part, *Specific Disorders and Syndromes*, begins with general approaches and tools that are applicable across disorders. These include attention to the whole child, rather than merely her symptoms; the importance of careful diagnostic assessment and measurement of symptom severity and paying close attention to the child's personal experience of being ill and treated with medication. The section encompasses a broad range of clinical populations, including the vulnerable and traditionally "orphan" ones in research and practice: very young children, and those with comorbid mental retardation, medical illness, or severe substance abuse. Each of the disorder-specific chapters is in turn related back to the underlying developmental psychopathology and drug-specific principles previously presented. Our goal has been to achieve the most integrated flow possible throughout the book. In so

doing, we have additionally hoped to emulate Donald's uncanny ability to move seamlessly between basic research and the care of vulnerable, suffering children.

Even if its shortest, the book's final part, *Epidemiological, Research, and Methodological Considerations*, is quintessential Donald Cohen in that it highlights the moral and scientific imperative *to know* that is necessary to be our most clinically effectively selves. In Donald's view, every single clinical encounter is, or should be, research. The ethical quandary for him was not in whether to do research in children, but rather in the *not* doing of it instead. As a mover and shaper of policy, he devoted much of his energy to increasing funding to bolster these activities at the local and Federal levels, as well as internationally. Given this last point, the volume appropriately ends with a chapter on international perspectives of pediatric psychopharmacology.

The success of NMI (whose second edition is well under way) reflects and contributes to the exciting developments rapidly taking place in the field of biological psychiatry. We also appreciate that part of such success can be attributed to the editorial excellence of Oxford University Press. We too are excited about the rapidly paced developments that propel our field each day, grateful to the volume's authors for their engagement and commitment to this work, and indebted to Fiona Stevens at Oxford for her superb and patient editorial support. More than anything, we are profoundly grateful and indebted to Donald Cohen, our mentor and friend. We miss him dearly, and hope in some modest way to celebrate his life and his gifts through this volume, our own posthumous gift back to him.

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LAWRENCE SCAHILL, PhD, MSN
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I

BIOLOGICAL BASES OF PEDIATRIC PSYCHOPHARMACOLOGY

> *[A] focus on the interplay between normal and atypical development, an interest in diverse domains of functioning, and an emphasis on the utilization of a developmental framework for understanding adaptation across the life course are among those elements that are integral to a developmental psychopathology approach.*
>
> D. Cicchetti & D.J. Cohen, 1995. Perspectives on developmental psychopathology (p. 3). In *Developmental psychopathology, volume I – Theory and methods*
> D. Cicchetti & D.J. Cohen, eds. New York: John Wiley & Sons, Inc.

The book's first section is itself subdivided into three main parts. The first of these, *Developmental Principles of Neurobiology and Psychopharmacology*, starts with an overview of brain development that highlights molecular mechanisms involved in this lifelong process and to their relevance to pediatric psychopharmacology. The two chapters that follow describe molecular mechanisms involved in drug action, starting at the synaptic cleft and moving through multiple messenger cascades into the heart of the cell's genetic machinery. The last two chapters are devoted to pharmacokinetics—the body's handling of alien molecules—with particular and clinically relevant attention to drug interactions.

A short intermediary section, *Genetic Principles*, provides an overview of those mechanisms set in downstream motion following psychotropic drug engagement, and which are ultimately responsible for their ensuing end-organ effects. The chapter on pharmacogenetics provides an up-to-date account of progress in this emerging field, and offers a glimpse ahead at one of the most exciting areas of pharmacology—one that may ultimately allow a more rational approach to medication choice for individual patients.

The third part, *Developmental Psychopathology*, delves into detailed disease-specific overviews. Each of the chapters covers issues pertaining to nosology and classification, to genetic determinants, brain systems implicated, environmental influences, and nature–nurture interactions. Neurotransmission and neuromodulation, hormonal and other developmental influences are addressed and whenever available; and relevant animal models are incorporated into the discussion. The interplay of normative and derailed development is a core concept for these chapters. Of the section's 12 chapters, nine are devoted to traditionally defined disease categories, and three cover the overarching areas of early-life stress, aggression, and affiliative behaviors.

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Developmental Principles of Neurobiology and Psychopharmacology

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1

Overview of Brain Development

FLORA M. VACCARINO AND JAMES F. LECKMAN

> What no one could have anticipated, however, are the implications for psychotherapy of the new evidence we have for neurogenesis and plasticity in the brain throughout life (Gross 2000; Shin et al. 2000). Every day, thousands of new neurons are added to the adult brain, some of them in circuits known to be crucial for learning and memory. Their actual number is small in proportion to the total population of neurons, but they may function in learning and memory by permitting the development of new connections as well as modulating older ones. Psychotherapy may literally alter brain structure by altering brain function. Eisenberg (2001, p. 745)

For most species, the nervous system is the most complex part of the organism. About 30,000–40,000 protein-coding genes exist in the human genome, over one-third of which are expressed in the central nervous system (CNS) (Lander et al. 2001). The reason for this abundance in gene expression lays in the intrinsic complexity, both structural and functional, of the CNS. It is reasonable to assume that CNS-specific transcripts are necessary to encode the wide variety of molecular components of the mature CNS; moreover, an even higher number of transcripts guide CNS morphogenesis and are primarily expressed during its formation. In this chapter, we introduce the reader to some of the basic rules that control CNS development and discuss how the workings of this genetic machinery are dependent on cell-to-cell communication and variations in the environment. Throughout this chapter, we will draw parallels between CNS development and aspects of the function of the mature brain to illustrate the conservation of mechanisms regulating brain plasticity throughout life. We also explore those ontogenesis of neurotransmitter and neuromodulatory systems that are targets of many of the pharmacological agents used in the pediatric age range. We have deliberately chosen examples to illustrate specific principles of CNS development of particular significance for the understanding of developmental psychopathology. For more complete and systematic accounts, the reader is encouraged to consult one of a growing set of volumes on this topic (Kandel et al. 2000).

GENES THAT SHAPE THE CENTRAL NERVOUS SYSTEM

The ontogenetic development of the CNS is driven by hierarchical sets of evolutionarily conserved genes. Although the sequence of gene expression that controls neural development is preset and stereotyped, it is also driven to a large extent by local interactions among developing cells. The evolutionary advantage of these interactions is that complex systems are able to reassess cellular identities and make appropriate adjustments to environmental perturbations. This is particularly true at the earliest stages of embryonic development, when the need exists to coordinate reciprocally the layout of different tissues and compartments. Regulatory interactions allow higher organisms to use alternative genetic pathways to adjust to environmental fluctuations; the system thus becomes more flexible and less prone to failure. As the genetic blueprint unfolds, cell fates become increasingly autonomous and less sensitive to variations in the environment.

Development involves the proliferation of cells to generate a multicellular organism, differentiation, and sorting of these cells into appropriate patterns. Pattern formation involves a series of cell fate decisions and the proper positioning of the progenitor and differentiated cells. These decisions are arranged in a hierarchy of

choices, where the simplest (i.e., axis formation) prefigures and regulates the more complex, such as the location of different neuronal types.

During CNS patterning, cells reciprocally coordinate their replication rate, fate, and relative position. These interactions are mediated by direct cell-to-cell contacts, by the local action of secreted polypeptides called *morphogens*, and by hormonal influences from the macroenvironment.

Morphogens are "form-generating" substances, whose configuration within a tissue "prefigures" the pattern (Meinhardt 1983). These substances set up an extracellular concentration gradient, which in turns orchestrates a coherent set of cellular behaviors that will eventually result in the proportionate growth of an organ, including the finest details. For example, different scalar concentrations may specify the type of cells and their relative position within the field. Morphogens act by altering the pattern of gene expression in target cells, because different genes, whose activation depends on different thresholds, will be turned on at different distances from the morphogen's source.

Specific examples can be found from the earliest stages of development. In *Drosophila,* before egg fertilization, localized morphogens present in the egg cytoplasm—the products of "maternal effect genes"—direct early pattern formation. Four maternally derived signals are responsible for the formation of the principal body axes—the anterior–posterior, the dorsoventral, and the terminal. These are established independently and provide a pattern for subsequent development (St. Johnston & Nusslein-Volhard 1992). One of the maternal signals, the *Drosophila*'s homologue of the epidermal growth factor (EGF), establishes the dorsal side of the fertilized egg by binding to its receptor, the EGFR. In mammals, EGFR regulates the development of the cerebral cortex and the proliferation of stem cells in the adult mammalian forebrain (Sibilia et al. 1998). In the absence of EGFR signaling, the cerebral cortex undergoes progressive degeneration, which suggests that this receptor is essential for cortical neuron survival. This example illustrates the striking conservation of molecular signals across development, often used with disparate functional outcomes.

Another maternal signal in *Drosophila* is the transcript of a gene called *bicoid*, which is present only at the anterior end of the egg (Fig. 1.1). The bicoid gene product is transcribed in an anterior-to-posterior gradient, highest at the anterior pole of the embryo. Bicoid is a homeodomain transcription factor that binds the regulatory region of zygotic genes, named *gap* genes, and turns them on. These genes, in turn, control the development of the head. Since bicoid binding sites on different gap genes have different threshold affinities for bicoid, the concentration gradient of this morphogen defines different areas of gene expression along the embryo (Fig. 1.1).

Gap genes are activated in discrete blocks that segmentally organize the body plan; as their name suggests, their inactivation results in the creation of a missing part in the developing embryo. For example, orthodenticle (*otd*) and empty spiracles (*ems*) are activated by high concentrations of *bicoid* in partially overlapping regions of the insect head (Finkelstein & Perrimon 1990). *Otd* and *ems* are required for the specification of the first, second, and third segments of the insect brain. Even though a segmental organization is much less obvious in the vertebrate CNS, gap genes are evolutionarily conserved and exert a similar function in higher species (Fig. 1.1). The mammalian homologues of *otd* and *ems, Otx1/Otx2* and *Emx1/Emx2*, are expressed in partially overlapping domains of the mammalian forebrain (Simeone et al. 1992; see the next section).

SELECTOR GENES CREATE REGIONAL DIVERSIFICATION

The orderly sequence of gene expression during subsequent development divides the embryo into units called *compartments*. Because cells belonging to one compartment do not mix with cells of other compartments, the founder cells of one compartment share a "genetic address" given by the expression of a unique combination of homeodomain selector genes. *Homeodomain genes* are master regulators of gene transcription, present in all eukaryotes (Krumlauf 1992). By binding to gene promoters through a conserved DNA-binding motif, the homeodomain (Gehring et al. 1994) selector genes give to the cells of one compartment a set of specific instructions that define their identity.

Hox genes are homeodomain selector genes that regulate segmentation in the vertebrate neural tube, including the spinal cord and the rhomboencephalon (Lumsden & Krumlauf 1996). Loss-of-function mutations of *Hox* genes result in growth abnormalities, deletions of rhombomeres, and alterations in cellular identities, leading to cell intermixing and axonal pathfinding defects (Capecchi 1997). Embryonic defects produced by thalidomide or retinoid toxicity have been attributed to misregulation of homeobox genes in the hindbrain and forebrain. Interesting parallels can be drawn between mouse mutants lacking *Hoxb2* and *Hoxb1,* in which the facial motor neurons fail to be specified, and the autistic features of Moebius syndrome (Gillberg & Steffenburg 1989; Goddard et al.1996). Early developmental defects in the rhomboencephalon possibly caused by Hox gene abnormalities have been hypothesized to occur in some cases of autism (Gillberg & Steffenburg 1989; Rodier et al. 1996).

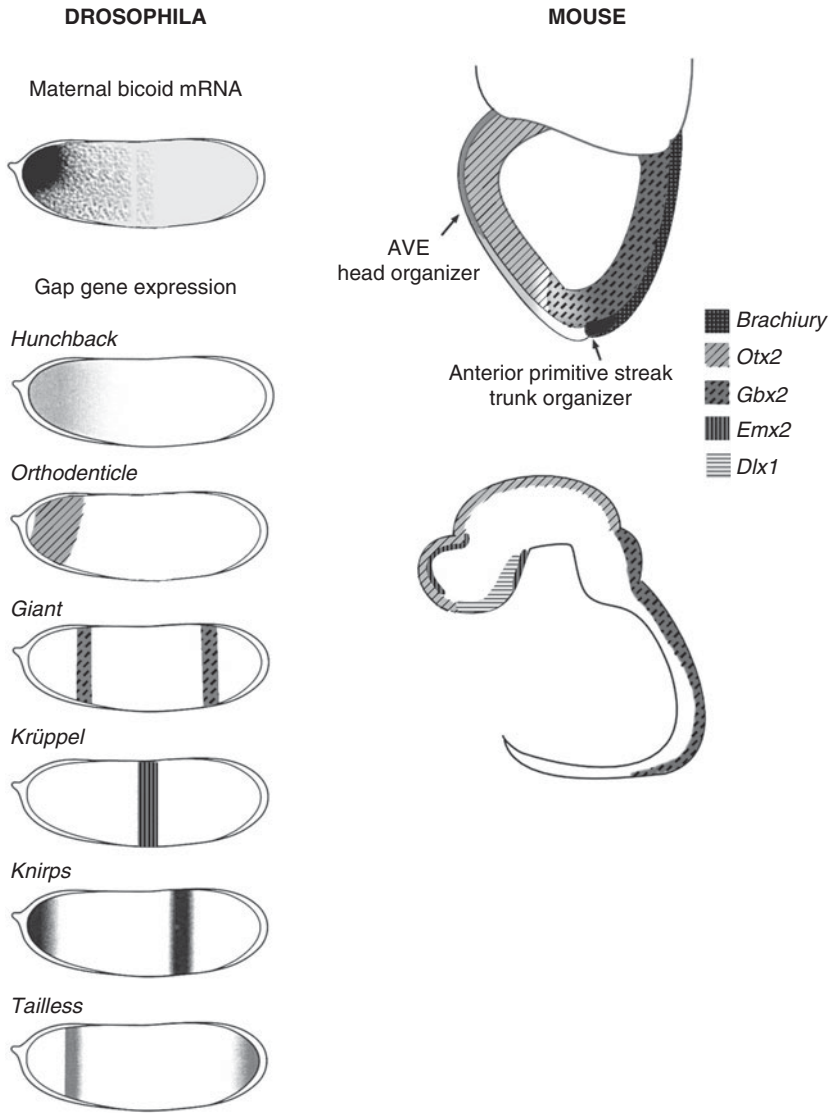

FIGURE 1.1 **The earliest genes that establish an anteroposterior pattern within the central nervous system (CNS).** *Drosophila's* early development is shown on the left. In *Drosophila*, anteroposterior patterning begins in the egg through maternally expressed genes such as *bicoid*. Gap genes are directly or indirectly the targets of *bicoid* and other maternally expressed morphogens. Anteroposterior patterning in vertebrate development (shown on the right) begins just before gastrulation. At this stage, the mammalian embryo is shaped as a cup. Two signaling centers, the head and the body organizers, are situated in the anterior visceral endoderm (AVE, head organizer) and the anterior primitive streak (anterior primitive streak trunk organizer), respectively. The AVE expresses the homeobox genes *Otx2, Hesx-1*, and *LIM-1*. The body organizer expresses *LIM-1, goosecoid, brachiury,* and *HNF-3B*. Genes within the head and body organizers directly or indirectly result in the transcription of *Otx2* and *Gbx2* in the neuroectoderm, which later specify the anterior and posterior CNS, respectively.

Several families of homeodomain genes expressed in the anterior part of the CNS (the prosencephalon) have now been described (Fig. 1.2). Their function has been determined in the mouse by gene inactivation studies.

The inactivation (knockout) of the *Otx2* gene by homologous recombination results in a failure of development of the whole prosencephalon (Acampora et al. 1995). This is the result of an apparent failure of primary regionalization of the CNS. *Otx1*, a close *Otx2* homologue, is important for development of the cerebral cortex. In the absence of *Otx1*, the cerebral cortex is smaller and has an abnormal lamination; as a result, mice develop intractable seizures (Acampora et al. 1996). Whereas a complete lack of function of *Otx* genes can result in an anencephalic phenotype, a partial lack of function of these genes causes a lack of midline structures and the partial fusion of the two lateral ventricles, a relatively common human malformation called *holoprosencephaly*. Although most forms of holoprosencephaly are lethal, milder forms are compatible with life.

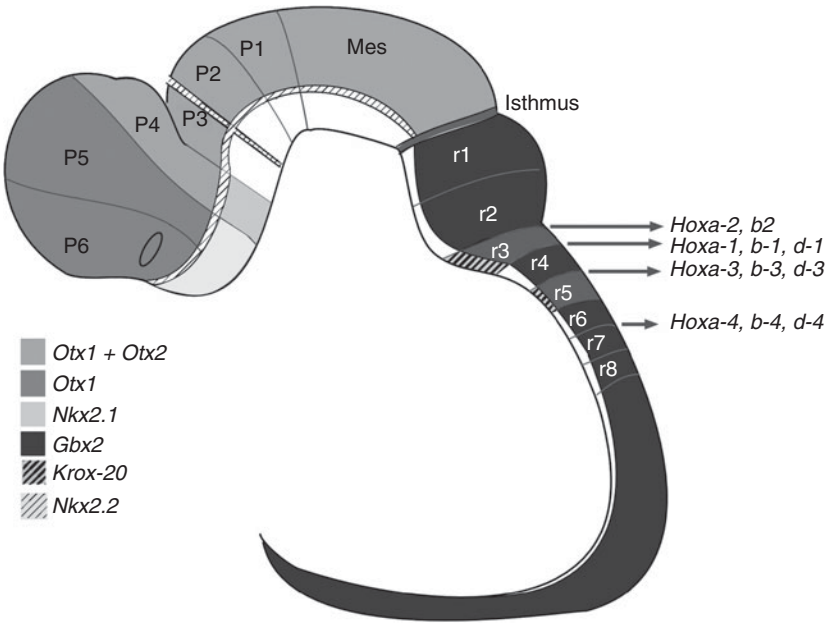

FIGURE 1.2 **The prosomeric model of forebrain patterning, defined by gene expression boundaries.** Schematic representation of the expression of *Gbx2, Nkx2.1, Nkx2.2, Otx1, Otx2,* and *Krox-20* in the mouse embryonic neural tube (embryonic day 11.5). The upper limits of expression of *Hox* genes in the brain stem are indicated by arrows. For more details, see Simeone et al. 1992; Rubenstein et al. 1994; Bulfone et al. 1995; and Shimamura et al. 1995.

Emx genes are important for the genesis of the hippocampus (Pellegrini et al. 1996), and point mutations of *Emx2* are associated with schizencephaly (Brunelli et al. 1996). Thus, these homologues of early segmentation genes in *Drosophila* serve fundamental functions in the development in the human brain.

ORGANIZER REGIONS ORIGINATE LONG-RANGE MOLECULAR SIGNALS

Organizers are groups of cells that lead to the generation of a new structure in surrounding tissue. These actions at a distance are mediated by the release of diffusible factors (morphogens) by cells within the organizer that induce cascades of gene expression in distant cells.

In the spinal cord, two morphogens acting in a contrasting manner, Sonic hedgehog (Shh) and bone morphogenetic protein (BMP), are generated along opposite sides, the ventral and dorsal neural tube. Shh diffuses within the ventral tube and specifies as many as five distinct progenitor domains, which in turn will give rise to distinct neuronal groups, such as motor neurons and ventral and dorsal interneurons (Briscoe et al. 2000) (Fig. 1.3A,B). Conversely, BMP4 is present in the dorsal midline, and it induces sensory interneurons in the dorsal spinal cord (Liem et al. 1995).

These diffusible ligands specify the fate of progenitor cells by modifying the expression of specific transcription factors. For example, *Shh* increases the expression of *HNF3* and *Nkx 2.1* and down-regulates *Pax3* and *Msx1*, resulting in a rapid disappearance of these genes from ventral regions (Hebert & Fishell 2008). Once established, these transcriptional domains stabilize and maintain their expression by reciprocal cross-repressive interactions and possibly through other mechanisms that are independent of downstream components of the *Shh* pathway (Jenkins 2009).

The isthmus, located just above the brainstem, is a constriction that delimits mesencephalon from rhombencephalon. At this boundary, *Otx2* and *Gbx2* expression abuts. These genes are involved in the differentiation of the brain and spinal tube, respectively. Several morphogens are expressed at the isthmus, notably *Wnt-1*, as well as several members of the fibroblast growth factor (FGF) family, including *Fgf8, Fgf17,* and *Fgf18* (Fig. 1.3A). A recent experiment has shown that a localized source of *Fgf8* introduced in the caudal diencephalon (P2) is capable of inducing a second midbrain in this forebrain location, which is in a mirror-image orientation with respect to the normal midbrain (Crossley et al. 1996). The second midbrain is flanked by a rudimentary cerebellum; this suggests that the ectopic source of *Fgf8* results in a new, isthmus-like organizing center. This respecification occurs because *Fgf8* induces in the forebrain genes typical of the midbrain/hindbrain boundary, such as *Gbx2*, while repressing forebrain genes such as *Otx2*. Because *Otx2* and *Gbx2* reciprocally antagonize each other, any decrease in *Otx2* expression produces an expansion of

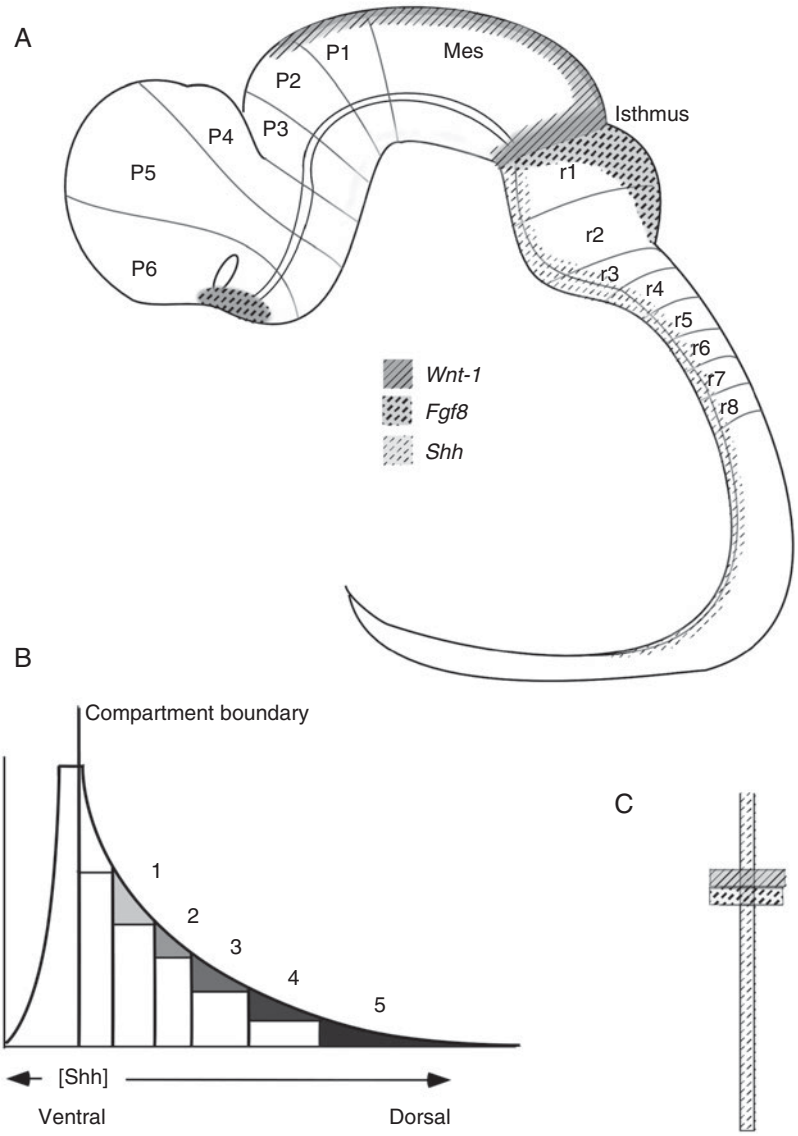

FIGURE 1.3 **Morphogens within the central nervous system (CNS). A:** Schematic view of a mouse embryo at approximately embryonic day 10.5–11.5 showing the distribution of *Wnt-1, Fgf8*, and *Sonic hedgegog (Shh)*. These morphogens are involved in the determination of neuronal fates by forming gradients along the anteroposterior and dorsoventral axes. **B:** The shape of Shh gradient from ventral (highest concentrations) to dorsal (lowest concentrations) is related to the determination of different progenitor pools along the dorsoventral axis of the neural tube. Because target genes may respond at different thresholds of *Shh*, progenitor fields will subsequently differentiate into specific neuronal types. Subsequently, reciprocal inhibitory interactions among the genes sharpen the boundaries. **C:** The intersection between two or more morphogens may be needed for the specification of neurons in particular loci within the CNS. For example, the location and number of aminergic progenitors may be specified the intersection of *Shh* with *Fgf8* at precise positions in the CNS.

the *Gbx2*-positive area and a repositioning of the isthmus (Acampora et al. 1997). The crucial role of *Fgf8* for midbrain/hindbrain development is confirmed by the fact that caudal midbrain and rostral hindbrain regions are absent when *Fgf8* is deficient (Meyers et al. 1998). In addition, *Shh* secreted from cells along the ventral midline of the brain intersects FGF ligands (Fig. 1.3A,C). Their cooperation triggers the development of dopaminergic and serotoninergic cells (Ye et al. 1998). The development of these cell types may be altered through subtle variations in these interactions among Shh and FGFs (Ohmachi et al. 2003; Saarimaki-Vire et al. 2007). These mouse studies directly informed studies in which investigators used *Shh* and FGFs to derive dopaminergic neurons *in vitro* to be used for cell therapy in Parkinson disease (Kim et al. 2002; Zeng et al. 2004). Furthermore, mutations in these neurotransmitter systems and their upstream regulators induced in mice serve as models to study the role of dopaminergic and serotonergic systems in affective disorders, psychoses, cognitive abnormalities, and autism. For example, mice that are deficient in serotonin 1_A

receptor during early postnatal development display heightened anxiety (Gross et al. 2002; Ramboz et al. 1998). Also, mice harboring knockouts for the dopamine receptors D_2 manifest spatial memory defects and other cognitive abnormalities (Gerlai et al. 2001; Glickstein et al. 2002). The abnormal behavior in these mice is mediated by altered development of forebrain systems, namely the cortex, basal ganglia, and hippocampus, that are targeted by these monoaminergic neurotransmitters.

These data suggest that neurotransmitters synthesized within the hindbrain and midbrain can exert long-range effects into the forebrain during development. In addition, short-range signaling systems act locally within the forebrain to regulate forebrain regional identity in a manner similar to that described for the hindbrain and spinal cord (Rubenstein & Shimamura 1997). For example, *Fgf8, Fgf18,* and *Fgf17* are expressed in the commissural plate, a signaling center at the rostral end of the brain, where they influence the development of the olfactory bulbs and dorsomedial cerebral cortex, namely, the prefrontal cortical areas. Intriguingly, the size of the dorsal prefrontal cortex is reduced in mice with a null mutation in *Fgf17* (Cholfin & Rubenstein 2007), and these mice concomitantly show altered social behavior (Scearce-Levie et al. 2008). Furthermore, mice lacking the FGF receptor gene *Fgfr2*, a receptor for Fgf17, exhibit a reduction in volume and total neuron number in the medial prefrontal cortex and altered development in downstream stations of the limbic system (Stevens et al. 2010).

DIVERSIFICATION AND EXPANSION OF CEREBRAL CORTEX DURING THE COURSE OF EVOLUTION

After the initial regional specification of the CNS, each region achieves further definition through evolved mechanisms for the control of its overall growth and for the differentiation of appropriate numbers of the indigenous cell types. The cerebral cortex has undergone a considerable expansion in its surface area during phylogenesis. The surface area of the human cortex is 1,000-fold larger than that of the mouse and 100-fold larger than that of the monkey. This increase in surface area is not matched by a corresponding increase in thickness (the human cortex is only three-fold thicker than the mouse) (Rakic 1995). Thus, the fundamental unit of the cortex (i.e., cortical column) has remained substantially the same during the evolution of the mammalian species, but the number of these units has increased. Second, there should be a mechanism operative during ontogenesis that leads to an increase in cortical surface area while maintaining the correct proportion of the different neuronal types.

Cortical neurons are generated in a layer of cells situated around the embryonic cerebral ventricles, the *pseudostratified ventricular epithelium* or *ventricular zone* (VZ). Progenitor cells within this layer proliferate and, after their final mitosis, leave the VZ and start migrating toward the primordial cerebral cortex (cortical plate). Although cortical areas differ, the pattern of neurogenesis appears to be similar throughout the cerebral cortex. The mechanisms that control the emergence of diversity between cortical areas may be intrinsic to the progenitors (i.e., there may be a protomap present within the ventricular neuroepithelium). Alternatively, the incoming afferent population may account for the generation of these regional differences while maintaining a fundamental identity in the cellular components of the cortex (Shatz et al. 1990). However, the VZ and emergent cortical wall contain gradients of transcription factors, including the homeodomain genes *Emx2, Pax6,* and *COUP-TF1,* as well as growth factor receptors like *Fgfr1*, *Fgfr2*, *Fgfr3*, early in embryogenesis, much before the ingrowth of thalamic afferents. Thus, it is now clear that the embryonic cerebral cortex acquires regional characteristics early in development, both in rodents and in the rhesus monkey. Furthermore, enhancing or inhibiting the expression of *Emx2*, which is expressed in a high posteromedial to low anterolateral gradient, results in a relative reduction or expansion of anterior cortical areas, respectively, suggesting that the EMX2 transcription factor represses rostral fates (Hamasaki et al. 2004). Similarly, knocking out *COUP-TF1*, which is expressed mostly in posterolateral sensory areas (parietal and occipital cortices), results in a massive expansion of frontal areas, including motor, to occupy most of the neocortex, together with marked compression of sensory areas (Armentano et al. 2007). In contrast, a decrease in FGF ligands that are emanating from the commissural plate, namely Fgf8 and Fgf17, results in a relative contraction of anterior (frontal) areas and parallel expansion of posterior cortical areas (Cholfin & Rubenstein 2007; Fukuchi-Shimogori & Grove 2001; Huffman et al. 2004). Thus, cortical organization is regulated by a process whereby morphogens and signaling molecules secreted by patterning centers positioned at the periphery of the cortical plate induce graded expression levels of transcription factors in cortical progenitors across the cortex. These factors then activate or repress fate determination genes that regulate area identities, and reciprocally cross-regulate their expression levels (O'Leary et al. 2007; Shimogori et al. 2004).

The expansion of founder cells that are originally specified to the cortical field directly affects cerebral cortical size. It has been postulated that the founder population expands through symmetric divisions before neurogenesis begins (Rakic 1995), whereas a predominant asymmetric mode of division underlies the generation

of neurons (Fig. 1.4B). The molecular mechanisms of asymmetric division are likely to be similar to those controlling the formation of compartments—that is, the differential inheritance of selector genes between the two daughter cells (Jan & Jan 1995). It is likely that cell death may also regulate the number of cortical founder cells, because mice lacking cell death effector molecules have profound abnormalities in brain anatomy (Roth et al. 2000). The time course of morphogenetic cell death in the developing cortex is the subject of intense investigation (see section "Large Numbers of Neurons Naturally Die During CNS Maturation" later in this chapter).

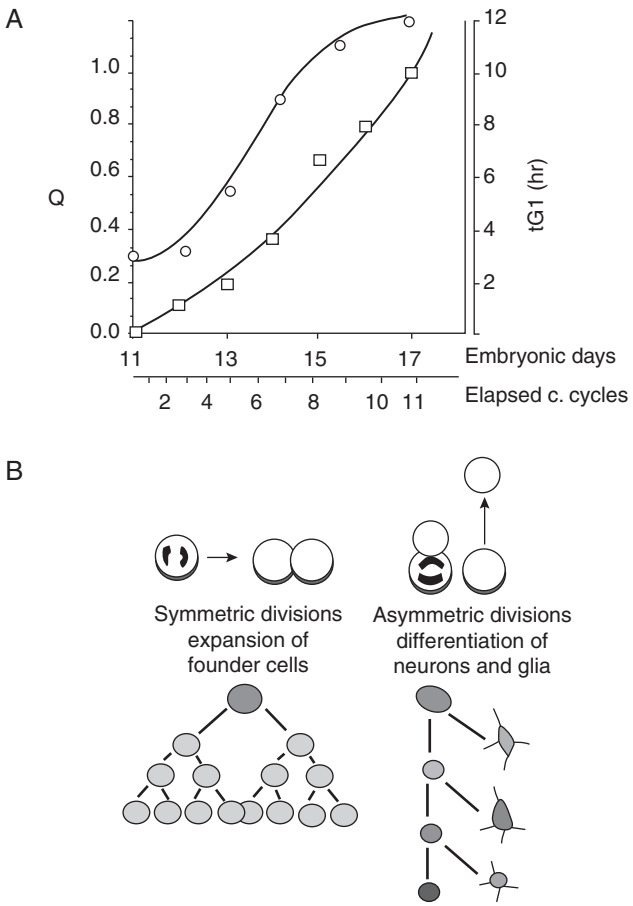

FIGURE 1.4 **A:** The progression of Q through cortical neurogenesis. Schematic time course of the proportion of cells that quit the cell cycle (Q, *squares*), and of the length of G1 (*circles*) during cerebral cortical neurogenesis. *Q* and G1 are plotted as a function of the cell cycle of the neurogenetic interval in the mouse cerebral cortical wall. The pseudostratified ventricular epithelium expands before Q = 0.5 (corresponding to embryonic day 14, as neurons of layer IV are forming) and contracts afterwards. Adapted from Takahashi et al. 1996; Miyama et al. 1997. **B:** Symmetric and asymmetric divisions. Symmetric divisions generating mitotic cells will exponentially increase the progenitor cell population, whereas asymmetric divisions generating a mitotic and a postmitotic daughter will keep the progenitor population at steady-state levels. The progenitor cells exhibit time-dependent changes in fate, giving rise first to neuronal and then to glial progeny (shown as a progressive change in gray shading).

Cortical progenitor cells undergo a defined number of cell cycles to generate the cerebral cortex—11 cell cycles occur in the mouse. The cell cycle length increases with the progression of neurogenesis because of a lengthening of the G1 phase (i.e., the phase of the cell cycle in which cells are quiescent and most RNA and protein synthesis occurs) (Fig. 1.4A). Takahashi et al. (1996) evaluated the kinetics of growth of the progenitor population by measuring the fraction of progenitor cells that exit the cycle (the *Q* fraction). *Q* increases in a nonlinear fashion through mouse neurogenesis, from 0 at the beginning (when all the cells are proliferative) to 1 at the end (when all the progenitor cells exit the cycle) (Fig. 1.4A). After embryonic day 14 ($Q < 0.5$), the rate of cells leaving the proliferative population exponentially increases, resulting in the contraction of the VZ and a massive generation of cortical neurons. Neurons populating the dense upper cortical layers are formed during the last 2 to 3 days of neurogenesis (Takahashi et al. 1996). During the last portion of neurogenesis, there is also an expansion of the subventricular zone (SVZ), a layer of dividing neuronal precursors that is situated above the VZ. The SVZ is a secondary population originally seeded from the VZ, and is particularly prominent in primates. The function of the SVZ may be that of generating upper-layer cortical neurons, as well as neuronal progenitors that will continue to be active in postnatal life (Dehay & Kennedy 2007; Martinez-Cerdeno et al. 2006; Molnar et al. 2006; Noctor et al. 2004).

The progression of the cell cycle is regulated through a checkpoint in early G1. Exposure to growth factors binding to receptor tyrosine kinases (RTK) during this phase leads to an early commitment of the cell to divide again, whereas growth factor deprivation in G1 leads to the degradation of D cyclins and to a failure to reenter the cycle (Ross 1996). Several FGF family members, including basic FGF (Fgf2), Fgf8, Fgf9, Fgf10, and Fgf15, are present in the developing cerebral cortex and are candidate mitogenic molecules that may affect the G1 checkpoint (Weiss et al. 1996). In contrast, EGF is active only later, and thus, EGF-responsive stem cells are the progeny of those that respond to Fgf2 (Tropepe et al. 1999; Vaccarino et al. 1995). The microinjection of Fgf2 in the embryonic cerebral ventricles increases the generation of cortical neurons; conversely, mice lacking a functional *Fgf2* have a decrease in the number of cortical cells and a decrease in the number of neuronal progenitors early in neurogenesis (Vaccarino et al. 1999; Fig. 1.5). Thus, Fgf2 is required for the attainment of an appropriate cell number in the cortex. The lack of Fgf2 has no effect on the length of the cell cycle, and thus Fgf2 is hypothesized to act by antagonizing cell cycle exit and thus regulating the expansion of the pool of cortical precursor/stem cells before and during neurogenesis (Raballo et al. 2000). Interestingly, Fgf2 is not necessary for either the development of the

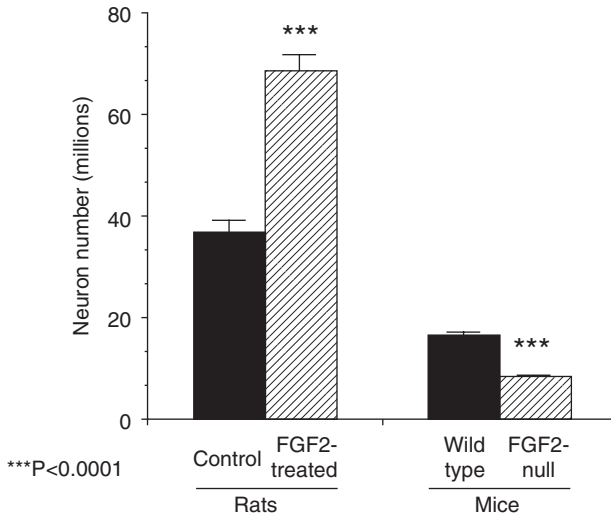

FIGURE 1.5 **Fgf2 is both sufficient and necessary for the development of a normal number of neurons in the cerebral cortex.** Adult control rats have approximately twice as many cortical neurons compared to wild-type mice. The number of cortical neurons doubled in rats that had received an Fgf2 microinjection during embryogenesis (Fgf2-treated rats). Conversely, mice with a germline disruption of the *Fgf2* gene (*Fgf2*–/–mice) had half the number of cortical neurons compared to wild-type mice. Total neuron number was estimated by stereological techniques on cresyl violet–stained sections. Significance was determined by ANOVA with Scheffé post-hoc test; ***p <0.0001.

basal ganglia or the generation of cortical interneurons that migrate into the cerebral cortex from the ganglionic eminences (Raballo et al. 2000). It is thus possible that fundamentally different mechanisms regulate the number of glutamatergic cortical pyramidal cells and the γ-aminobutyric acid (GABA)ergic cortical interneurons (see next section). Consistent with this role of Fgfs, mice with a conditional deletion of Fgf receptor genes display an almost complete absence or a reduction in size of the cortical primordium and curtailed excitatory neuron genesis due to failed maintenance of neural stem cells, the severity of which depends upon how early in development the deletion occurs (Paek et al. 2009; Stevens et al., 2010.

Another important mechanism regulating cortical precursor fate is the Notch signaling pathway, which include the Notch receptor and several ligands, including Delta1 and Jagged1. Based on a large number of invertebrate and vertebrate studies, the role of Notch is similar to that of the Fgf signaling pathway; that is, Notch inhibits neurogenesis and promotes stem cell self-renewal. The mechanism of Notch action is to antagonize the transcription of basic helix-loop-helix (bHLH) proneural transcription factor genes *Mash1* and *Neurogenin 2* (*Ngn2*) which induce the neuronal differentiation program (Kageyama et al. 2009). As cells differentiate, they activate *Mash1* and *Ngn2* expression, which in turn will increase the expression of Delta1 on their surface, and activates the Notch receptor on neighboring cells. After activation, the Notch intracellular domain is released and migrates to the nucleus, where it forms a complex with the DNA binding protein RBPj. The Notch-RBPj complex activates the transcription of *Hes1* and *Hes5*, which will repress the transcription of proneural genes and *Delta1*, leading to the maintenance of a precursor/stem cell state. In the cerebral cortex, as well as in other systems (i.e., somitogenesis), both Notch signaling and Fgf signaling dynamically oscillate in progenitors, and these oscillations appear to be regulated independently of each other (Aulehla & Pourquie 2008; Kageyama et al. 2008, 2009; Shimojo et al. 2008).

In summary, ligands for RTK and Notch are dynamically activated within microdomains of the VZ to regulate the timing of exit from the cell cycle, the number of progenitor cell divisions, and cell differentiation. These interactions occurring in an individual are likely to lead to variations in the number of glutamatergic and GABAergic neurons, as well as to glia born during this phase of CNS development.

NEURONAL MIGRATION IN THE CEREBRAL CORTEX: RADIAL AND TANGENTIAL

In laminar structures such as the cerebral and cerebellar cortices, glial cells of a specialized nature, which are the Bergmann glia and the radial glia for the cerebellum and cerebral cortex, respectively, guide young neurons in their radial migratory path. Another set of neurons migrates nonradially using unknown cellular and molecular pathways, possibly utilizing early axonal tract or other astroglial cells (see below).

In the developing cerebral cortex, cells of the marginal zone (the Cajal-Retzius cells) are the first to be born. Subsequently, neurons pile up underneath the marginal zone to form cortical layers, in the sequence 6-5-4-3-2. The youngest cells always penetrate and migrate past the last ones and occupy the area nearest the marginal zone.

Several human genetic mutations exist that disrupt neuronal migration and cortical layer morphogenesis. These cause an arrest of the migration of cortical neurons, which produces various degrees of mental retardation and seizures (Gleeson & Walsh 2000).

The analysis of several mouse mutants has given important insights into the complex signaling mechanisms that guide migrating neurons and has emphasized the importance of genetic components in the mechanism of cell migration. The *reeler, scrambler*, and *yotari* mutations in the mouse cause an identical phenotype in which younger migrating neurons are unable to penetrate the layer of older neurons. As a result, there is an

inversion of cortical layers and a consequent disorganization of the cerebral cortex and other laminated structures, such as the retina and cerebellum (Caviness 1982; Sheppard & Pearlman 1997). The *reeler* gene encodes for reelin, a secreted protein produced by Cajal-Retzius cells and densest in the marginal zone. The *scrambler* and *yotari* mutations disrupt components of the intracellular reelin pathway. Recently, it was found that the double null mutation for the apolipoprotein E (ApoE) receptor 2 and the very-low-density lipoprotein receptor (VLDLR) reproduces the reeler phenotype. This is because *reelin* binds to both ApoE2 and VLDLR with high affinity, and this binding transduces the *reelin* signal. Signaling through nonreceptor tyrosine kinases is critically involved in the regulation of neuronal migration.

A mutation in *reeler* has been associated with autism (Persico et al. 2001). Abnormalities in cortical neuron migration and in reelin-containing cortical interneurons have been observed in the brains of schizophrenic subjects (Guidotti et al. 2000). Therefore, a mutation in *reelin* has also been implicated in schizophrenia. Changes in reelin have also been reported in the hippocampus in affective disorders (Fatemi et al. 2000).

Glutamatergic pyramidal cells migrate in a radial fashion from the dorsal VZ, and are lineally distinct from GABAergic interneurons, which are tangentially dispersed. The origin of the tangentially migrating GABA neuron precursors are the ganglionic eminences, which are located in the ventral part of the telencephalon (Anderson et al. 2001; Fig. 1.6). Progenitor cells of the basal telencephalon are genetically distinct from those located in the dorsal VZ, as they express different sets of homeodomain genes, including *Dlx* and *Nkx* (Figs. 1.1 and 1.2), which are not shared by dorsal cortical progenitors. Although in mice most GABA neurons originate from the ventral telencephalon, in humans a portion seem to arise from the dorsal VZ.

The common origin of cortical and basal ganglia GABAergic cells and their dependence on a common set of genes necessary for their differentiation and migration pose interesting clinical questions. Deficits in cortical cell migration and in GABAergic interneurons have been observed in schizophrenia (Akbarian et al. 1996; Benes et al. 1996). Similarly, abnormalities in cortical and basal ganglia interneurons have been reported in Tourette syndrome and obsessive-compulsive disorder (Ziemann et al. 1997; Greenberg et al. 2000; Kataoka et al., 2010). These last conditions are thought to be disorders of the basal ganglia, and indeed, in Tourette syndrome, a prominent decrease in both cholinergic and GABAergic parvalbumin-containing interneurons was detected in the caudate and putamen (striatum). Interestingly, the strongest decrease in cholinergic neurons was present in the associative region of the striatum,

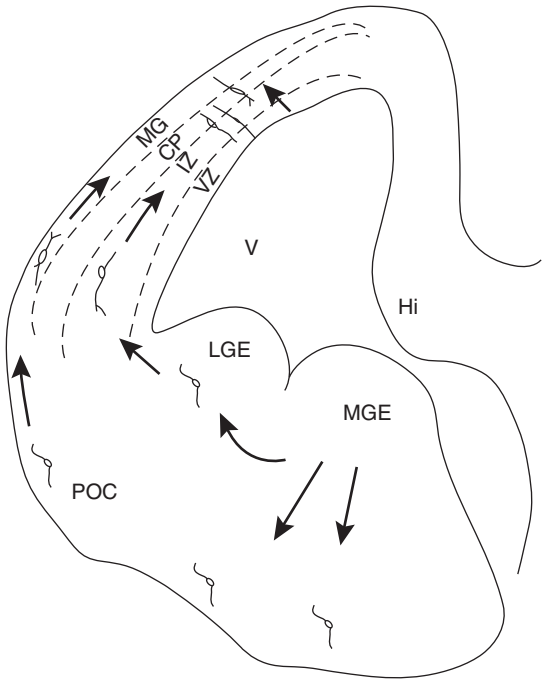

FIGURE 1.6 **Modes of cell migration to the cerebral cortex.** Schematic drawing of the cerebral cortical wall of the rodent at about embryonic day 13.5. The arrows indicate the main routes of cell migration. Abbreviations: CP, cortical plate; Hi, hippocampal formation; IZ, intermediate zone; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; MZ, marginal zone; POC, primary olfactory cortex; V, lateral ventricle; VZ, ventricular zone. Adapted from de Carlos, J.A., Lopez-Mascaraque, L., & Valverde, F., 1996. Dynamics of cell migration from the lateral ganglionic eminence in the rat. *Journal of Neuroscience* 16:6146–56, with permission of the publisher, Society for Neuroscience.

suggesting an abnormality of frontostriatal circuitry (Kataoka et al., 2010). It is now clear that events occurring during the development of the ganglionic eminences may influence the development of the cerebral cortex and the hippocampus as well, since the basal ganglia is the origin of cortical inhibitory interneurons.

LARGE NUMBERS OF NEURONS NATURALLY DIE DURING THE EARLY PHASE OF CENTRAL NERVOUS SYSTEM MATURATION

Neurons are produced in excess and are later eliminated by a process of natural cell death called *apoptosis*. The death of a cell is carried out by a program encoded by its own genome and is descriptively characterized by a stereotyped sequence, starting with shrinkage and breakage of the chromatin in the nucleus (Fraser & Evan 1996). This death program involves a common set of molecules conserved throughout evolution, beginning with primitive unicellular eukaryotes (Vaux & Strasser 1996). The reason for the conservation of cell

death programs throughout animal evolution may be that the ability of an organism (or of a colony of cells) to kill part of itself might provide a competitive advantage to the remaining cells. Microglia and astrocytes, the primary immunocompetent cells of the CNS, are critically involved in apoptosis, as well as in other aspects of brain development (Bilbo & Schwarz 2009). In general, cells eliminated by apoptosis are abnormal and/or potentially dangerous (i.e., cells that fail to follow the appropriate programs of division or differentiation: cancer cells, autoreactive lymphocytes, or virally infected cells). In addition, apoptosis plays a role in selecting neurons that have established unique patterns of signaling. This contributes to tissue sculpting during morphogenesis and the maturation of neuronal circuitry in the CNS.

In general, excess cells undergo apoptosis under conditions of trophic factor scarcity. For example, in sympathetic ganglia, neurons undergo cell death in early embryogenesis unless they are able to connect with their target, a source of nerve growth factor (NGF), for which several neurons compete. In the CNS, growth factors may be delivered to a neuronal cell body not only retrograde by the target but also anterograde by the afferents. Furthermore, neuronal activity may regulate the synthesis of growth factors, and patterns of activity in the CNS are not only triggered by afferent stimulation but are often a characteristic of the network (for example, reentrant circuitry).

Different cell populations die at different times. For example, in rodents, sympathetic neurons are pruned down by mid-gestation, whereas for cranial regions of the neuraxis, the phase of cell death extends into the perinatal period. In the mammalian cerebral cortex, it has been estimated that approximately 40%–50% of neurons die, and this process is completed in the rat by the third postnatal week, concomitant with synaptogenesis (Ferrer et al. 1990; Fig. 1.7).

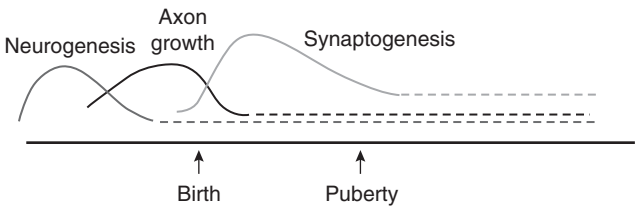

FIGURE 1.7 **Time course of neuronal and synaptic remodeling in the rodent cerebral cortex.** Neurogenesis and pruning of cells, axon collaterals, and synapses are distinct processes with partially overlapping time courses. Data are compiled from Fraser & Evan 1996; Vaux Strasser 1996; Ferrer et al. 1990.

INBORN GENETIC PROGRAMS CONTROL FORMATION OF NEURONAL CONNECTIONS, WHEREAS ACTIVITY REGULATES THEIR REMODELING

One of the most formidable tasks for neurons within the developing CNS is to successfully "find" the appropriate target with which to establish synaptic connections. In the adult human CNS, over a trillion neurons each connect with, on average, a thousand target cells according to precise patterns essential for proper functioning (Tessier-Lavigne & Goodman 1996). Rather than establishing random connections that are later shaped into the normal pattern of connectivity, from the very beginning neurons are endowed with the capability to "choose" appropriate targets. Different mechanisms underlie the directed growth of the axon, the recognition of the target, and the transformation of the growth cone into a synapse; these mechanisms appear to be independent of electrical activity (Goodman & Tessier-Lavigne 1997). The growth of axons toward the target is orchestrated by homeodomain proteins, which control the transcription of genes that regulate axonal pathfinding, differential cell adhesion, and synapse formation. The resulting pattern of connectivity is largely accurate and can be later refined by mechanisms influenced by electrical activity.

Growing axons "navigate" in the developing neuropil with the help of a sophisticated structure, the *growth cone*, and make few errors of navigation. The philopodia, dynamic web-like structures at the end of the growth cone of immature neurons, are able to "sense" or explore variations in the surrounding environment. These cues result in either axonal growth (attractive cues) or withdrawal and an abrupt turn in a different direction (repulsive cues). Attractive and repulsive cues are either diffusible (long-range) or nondiffusible (short-range) (Tessier-Lavigne & Goodman 1996). Once the target is finally reached, the growth cone stops and "transforms" into a synapse. Yet, the essential steps governing synapse formation are largely unknown.

Short-range cues generally involve the contact of axons with cell surface and extracellular matrix proteins. Adhesive substrates, or *cell adhesion molecules* (CAMs), are divided into three families; the cadherins, the integrins, and the immunoglobulin (Ig) superfamily. These transmembrane proteins possess a large extracellular moiety mediating adhesion and an intracellular portion that is linked to the cytoskeleton (Reichardt & Tomaselli 1991). Members of the Ig superfamily promote adhesions between cells by interactions among different members, such as neural CAMs (N-CAMs), L1, Down syndrome CAM (DSCAM), and axonin-1, expressed on the surface of adjacent cells. Their reciprocal binding is also influenced by their degree of glycosylation and their sialic acid content. Mutation of these proteins often causes embryonic defects in CNS morphogenesis, as cell adhesion is governing cell movement and layer formation, in addition to selective defects in axonal pathways. Examples are neural tube defects,

hydrocephalus, and callosal agenesis, but also defects in learning and memory, thus suggesting connectivity and synaptic alterations (Demyanenko et al. 1999; Kadowaki et al. 2007; Markram et al. 2007).

Diffusible (long-range) factors are soluble attractants or repulsants for growth cones; these are distributed in concentration gradients and aid selective growth in a particular direction. For example, cells at the ventral midline of the CNS express Netrins, which attract axons toward the midline in the formation of commissural connections (Serafini et al. 1996). Other long-range cues are provided by Semaphorins and their receptors Neuropilins. An important function of guidance molecule has been discovered in the process of interneuron precursor migration: the homeodomain Nkx2.1, which is expressed by GABAergic precursors in the medial ganglionic eminence and regulates their initial specification, also controls their migration by repressing the expression of Neuropilins. The embryonic striatum expresses the Semaphorins 3A and 3F, which act as chemorepellents for neurons expressing their receptor Neuropilins. GABAergic precursors migrating to the cortex down-regulate Nkx2.1, de-repressing Neuropilin-2, which will allow them to avoid the striatum and migrate into the cortex; in contrast, maintenance of Nkx2.1 will down-regulate Neuropilin and allow interneurons to migrate to the striatum (Marin et al. 2001; Nobrega-Pereira et al. 2008).

Ligands for RTK represent yet another class of long-range attractive cues. Retinal axons follow a downward gradient of FGF to enter the tectum (McFarlane et al. 1996), whereas an increasing gradient of NGF or NT3 is required for target recognition in other areas (El Shamy et al. 1996).

After reaching the target area, axons may have to be sorted according to a particular layer, or they must be arranged according to the original topographical information that they carry, such as in maps. A classic example is the arrangement of retinal axons in the tectum, which must reproduce a map of the retina. Experiments have shown that the retinotopic order is preserved even when size disparities are introduced, suggesting that selective affinity for molecular gradients are involved (Patterson & Hall 1992). Members of the ephrin (Eph) family of RTKs precisely orchestrate these cellular behaviors. Their membrane-anchored ligands are expressed as anterior-to-posterior gradients in the tectum and act as region-specific contact repellents (Tessier-Lavigne & Goodman 1996).

In a second phase, connections are rendered more precise by elimination of axons and synapses by activity-dependent processes during early postnatal development (Innocenti 1981). In rodents, this process extends into the first weeks after birth (see Fig. 1.7). In the fetal rhesus monkey, new axons start growing into the two major cerebral commissures (the corpus callosum and the anterior commissure) in the last weeks of gestation, with their growth peaking at birth. Axons are subsequently eliminated in the first 2 or 3 postnatal months at a precipitous rate. For example, during the first 3 postnatal weeks, axons are eliminated from the anterior commissure and from the corpus callosum at an average rate of 1 and 50 axons/second, respectively (LaMantia & Rakic 1994). The processes regulating this wholesale elimination are of interest, because the morphometry of the cerebral commissures correlates with a variety of behavioral differences, including gender, sexual orientation, and handedness (Allen & Gorski 1991).

Similar to axons, synapses are initially overproduced in the infant primate, reaching their maximum number during infancy (2–4 months of age) (Rakic et al. 1986). Cortical synapses are eventually pruned down to a density of approximately 15–20 synapses/100 μm^2 of neuropil. Axons and synapses are eliminated through different time courses (Fig. 1.7). In primates, the adult number of synapses in the primate cerebral cortex is not achieved until near adolescence (Rakic et al. 1986).

Axonal and synapse elimination occurs at the same time as growth of myelin, increase in size of neurons and glia, and other processes; this suggests that the mammalian brain may be considerably more plastic than previously thought. Recent large-scale magnetic resonance imaging (MRI) longitudinal studies of normal human development have revealed that both the white and gray matter of the cerebral cortex increase in volume during the postnatal period. For the cerebral cortical gray matter, the mean peak volume is reached at age 12 years, only to decrease thereafter. Remarkably, different cortical areas differ with respect to the age of peak growth, with the temporal lobe reaching a peak volume later (age 18 years) than the frontal or parietal lobes. The anterior portion of the corpus callosum increases until adolescence. Hence, increases in cortical volume occur throughout childhood, adolescence, and, sometimes, early adulthood (Giedd et al. 1999; Rapoport et al. 1999; Sowell et al. 1999).

Determination of the cellular and molecular processes that underlie these maturational events is under intense debate. Although the anatomical substrate of this growth is still unknown, the magnitude of these phenomena is such that it is likely to involve all components of the CNS, including neurons, glia, and their connections. The prevailing view has been that neurons are generated only during embryogenesis, and that their number decreases during the postnatal period through programmed cell death. More recently, these ideas have been challenged, as experiments in rodents, primates, and humans have shown that new neurons are normally generated in the hippocampus, olfactory bulb, and possibly other areas in postnatal brains (see section "Pluripotent Cells in Adult Forebrain May Be Involved in Regeneration").

Future research looking into the cellular basis of brain growth and remodeling from infancy into early adulthood is likely to shed light on the pathogenesis of disorders with onset in childhood and adolescence.

EXUBERANT CONNECTIONS ARE PRUNED OFF AS A CONSEQUENCE OF ACTIVITY-RELATED PROCESSES

Critical periods are those in which the synaptic circuitry of a given brain region becomes stabilized in a functionally optimized conformation. The cerebral cortex and other brain structures contain functional maps for the activities characteristic of a species. The best example is the ocular dominance columns and other physical and functional characteristics of the circuitry within the visual cortical system that process information derived from the left and right visual fields, producing a map of the visual world. Other internal maps exist for the body (somatosensory, motor cortices), the external physical environment (hippocampus), and possibly an individual social environment.

The primary visual cortex, like the rest of the cerebral cortex, is organized into vertical assemblies of neurons called *cortical columns*, which are the functional units of cortical information processing (Mountcastle 1957). The initial formation of these columns is independent of visual experience (Crowley & Kats 2000). In the primary visual cortex, separate cortical columns receive input from the right or the left eyes (ocular dominance). The maintenance of these connections is critically dependent on patterns of visual activity. If animals are deprived of visual input from one eye during the first 2–3 weeks after birth (the critical period), the cortical area occupied by columns for the functional eye enlarges at the expense of that for the deprived eye, which eventually becomes virtually unable to drive cortical activity (Wiesel & Hubel 1963). The effects are permanent for the life of that individual. This physical and functional "disconnection" of an inactive input occurs only during the critical period, as eye suture after the first weeks of life no longer affects cortical representation. One mechanism responsible for these effects is synaptic plasticity; generally, connections whose activity is temporally correlated are strengthened, whereas connections that display noncorrelated activity tend to be weakened. This phenomenon is apparently consolidated during sleep.

Critical periods may vary depending on the area of the brain and the activity involved. Despite the evidence that patterns of neural activity influence the organization of neuronal circuitry, the mechanisms involved remain elusive. Neuronal activity drives the selective survival and sprouting of branches, accompanied by the local addition of synapses, within appropriate areas; furthermore, the lack of activity promotes the pruning of synaptic connections from inactive areas (Katz & Shatz 1996). These competitive processes increase the refinement and precision of maps and require the activity of excitatory receptors (Antonini & Stryker 1993; Constantine-Paton et al. 1990) and locally released growth factors (Inoue & Sanes 1997; Thoenen 1995). In addition, there is a crucial role of the GABAergic system in driving both the onset and the termination of the critical period for plasticity, which may be ultimately mediated by altered regulation of *N*-methyl-d-aspartic acid (NMDA) receptors (Fagiolini & Hensch 2000; Hensch 2005; Kanold et al. 2009).

Changes in synaptic structure and strength are also thought to underlie learning. Long-term potentiation (LTP) and long-term depression (LTD) consist of increases or decreases in synaptic strength, which depend on previous patterns of activity. *N*-methyl-d-aspartate receptors and growth factors are also involved in LTP and LTD. In addition, structural changes (addition or removal of dendrites and spines) are thought to be involved in learning. Recent in vivo high-resolution imaging studies have shown that the overall morphology of axons, dendrites, and spines is remarkably stable in the adult CNS. Nevertheless, there is increasing evidence that experience-dependent plasticity of specific circuits involves cell type–specific structural plasticity: some boutons and dendritic spines appear and disappear, accompanied by synapse formation and elimination, respectively (Holtmaat & Svoboda 2009). Thus, similar or identical mechanisms are used during the development of synaptic connections and the remodeling of these connections during learning (Kandel et al. 2000).

PLURIPOTENT CELLS IN ADULT FOREBRAIN MAY BE INVOLVED IN NEURONAL REGENERATION

Most progenitors are lineage-restricted to produce either neurons or glia (Luskin et al. 1988). These progenitors have a limited lifespan (see Fig. 1.4B). Recent studies have shown that, in addition to fate-restricted progenitors, the forebrain contains pluripotent stem cells capable of differentiating into many different cell types, including neurons and glia (Weiss et al. 1996). A small number of these pluripotent cells persists in the subependymal zone of the adult brain in virtually every mammal that has been examined (Reynolds & Weiss 1992). These cells normally give rise to neurons that migrate to the olfactory bulb (Luskin 1993). Under normal conditions, neurogenesis does not seem to occur in either the adult striatum or the cerebral cortex, although apparently transient cortical cell genesis has been observed in the adult primate (Gould et al. 1999). However, neurogenesis has been observed in the juvenile mammalian

cerebral cortex, where the new neurons have been traced as the progeny of immature astrocytes (Ganat et al., 2006). The adaptive role of these glial cells present in the postnatal immature brain is highlighted by the fact that their proliferative and neurogenic potential is enhanced by injury (Fagel et al., 2006) a response that appear to be lost in the adult brain. Furthermore, the injury-mediated adaptive reaction in postnatal progenitors and stem cells is modulated by endogenous growth factors already known to play a role in embryonic neurogenesis, namely FGFs and their receptors (Fagel et al, 2006; Fagel et al., 2009). Rodent or human stem cells expanded *in vitro* in the presence of either Fgf2 or EGF and transplanted *in vivo* are able to populate various regions of the adult CNS, including the cerebral cortex, striatum, and substantia nigra (Fricker et al. 1999; Gage et al. 1995). Although the efficiency may be low, the new neurons appear to be perfectly integrated in the transplanted regions, suggesting that new neurons can be incorporated into the adult brain.

The adult hippocampus also contains progenitors/stem cells that are capable of differentiating into new hippocampal granule neurons. Hippocampal progenitor cells will give rise to new neurons in adult rodent, primate, and human brains (Eriksson et al. 1998). The new hippocampal granule cells extend axons that are appropriately connected to the pyramidal cells in the CA3 region and develop normal synapses over a period of about 1 month, after which they are fully integrated into the hippocampal circuitry.

The conditions that promote adult neurogenesis and the functional significance of adding extra neuronal cells to the adult synaptic circuit are presently not clear. It is clear, however, the both the hippocampal dentate gyrus and the olfactory bulb are regions in which a constant neuronal turnover, due to programmed neural cell death, occurs throughout life. The new neurons thus replace those that are normally lost. In contrast, neurons do not die in other brain regions except in the presence of pathology.

The conditions that promote neurogenesis have attracted considerable interest in psychiatry because neurogenesis is modulated by environmental conditions. For example, increased neurogenesis in rodents has been associated with exposure to an enriched environment, the performance of learning tasks, or simply running in a wheel (Kempermann et al. 2000). In contrast, stress strongly decreases the proliferation of adult neuronal progenitor cells in both rodents and primates (Gould et al. 1997). Cell damage or death promotes neurogenesis in the cerebral cortex, where neurogenesis does not normally occur in the adult (Magavi et al. 2000). These finding have generated considerable interest, since the theoretical possibility exists that new neurons could be generated to replace those lost to disease or degeneration (Weiss et al. 1996).

Hippocampal neurogenesis is enhanced by antidepressant drugs, suggesting that, in some ways, this process may be related to the pathophysiology of depression. Although it is clear that antidepressants increase neuronal progenitors cells and newly generated neurons in the dentate gyrus (Boldrini et al. 2009; Malberg et al. 2000), it is also likely that only some of the therapeutic actions of antidepressant treatment are dependent upon hippocampal neurogenesis (David et al. 2009; Santarelli et al. 2003). The link between neurogenesis and affective disorders is reinforced by the observation that, in depression and post-traumatic stress disorder, decreases in hippocampal volume occur that may reflect cell loss (Bremner et al. 2000; Sheline 2000). However, cell loss has been also found in the prefrontal cortex of depressed patients (Rajkowska 2000).

THE IMPACT OF GONADAL STEROIDS AND DEVELOPMENT OF SEXUALLY DIMORPHIC AREAS

Gonadal steroids act on the developing nervous system to create a variety of sex differences in neural organization. There are also marked sex differences in the incidence of certain neuropsychiatric disorders of childhood and adolescent onset that are likely influenced by these developmental mechanisms (female predominance: adolescent-onset depression and eating disorders; male predominance: autism, other autism spectrum disorders [ASDs], Tourette syndrome, and childhood-onset obsessive-compulsive disorder). Sexually dimorphic behaviors in invertebrate and vertebrate species have been linked to structural differences in the CNS (Allen & Gorski 1991; Goy & McEwen 1980). Although some of these effects are likely to be hormone-independent, gonadal steroids (estrogens and androgens) acting during the course of CNS development can influence the number, size, and connectivity of neurons in a variety of brain regions (Arnold & Gorski 1984; Balan et al. 1996; Pilgrim & Hutchinson 1994). For example, the increased size of the anteroventral periventricular nucleus of the hypothalamus in male rats appears to depend on the action of testicular hormones during the neonatal period, although the actual structural difference between the sexes is not obvious until puberty (Davis et al. 1996).

Intriguingly, for many areas of the brain, the action of testosterone depends on its conversion to estradiol by aromatase. The emergence of sexually dimorphic regions may depend on the creation of sex-specific networks of estrogen-forming neurons. For example, investigators have measured aromatase activity in two strains of mice selectively bred for behavioral aggression. The animals bred to have short attack latency showed a different developmental pattern of aromatase

activity in both the amygdala and the hypothalamus (Hutchinson et al. 1995).

Traditionally, investigators have focused on the role of steroid receptors acting via the nucleus and the binding of the steroid–receptor complex to specific DNA regions to alter the transcription of specific genes (Evans 1988). More recent studies have indicated that estrogen may also act through effects on the signaling pathway of NGF to induce changes in dendritic arborization and synapse formation. For example, ovariectomized female rats lose dendritic spines in specific hippocampal regions. When treated with estrogen, these animals show a 30% increase in NMDA receptors in the same hippocampal regions (Gazzaley et al. 1996; Wooley et al. 1997).

FUTURE PROSPECTS: ALTERATION OF NEURONAL DEVELOPMENT AND VULNERABILITY TO PSYCHOPATHOLOGY

The past decade has seen unprecedented advances in our understanding of the mechanisms involved in the morphogenesis and activity-mediated sculpting of brain circuitry. The reciprocal interplay of conserved genetic programs and the ever-changing macro- and microenvironment is a recurrent theme. These events set the stage for individual differences and range of phenotypic diversity seen within the human species (Bartley et al. 1997). A deeper understanding of these mechanisms should lead the way to improved treatments and preventive interventions.

Many psychopathological states, such as schizophrenia, autism, or Tourette syndrome, are fundamentally developmental disorders that likely involve allelic variants that confer vulnerability to specific environmental risk factors (Ciccheti & Cohen 1995). A developmental perspective is of value in considering childhood-onset disorders and will likely prove to be broadly useful. For example, the ability of estrogens to maintain a rich dendritic arborization in regions of the hippocampus may herald an effective means of maintaining mental function and preventing the toxic effects of substances such as β-amyloid (Tang et al. 1996).

In addition, gene programs that are instructing the development of the CNS may be "reactivated" at later stages, in connection with brain plasticity that characterizes learning and adaptation (Vaccarino et al. 2001; Stevens et al, 2010). However, we need to enlarge our perspective and the breadth of our studies to include genetic, immune, and developmental mechanisms that are primate-specific and even human-specific (Johnson et al. 2009; Ziv & Schwartz 2008). We predict this effort will identify the peculiarities that distinguish brain development in humans, both in terms of genetic regulatory processes and cellular events, and how these events may be deranged in specific neuropsychiatric disorders.

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2

Synaptic Function and Biochemical Neuroanatomy

STEPHAN HECKERS, CHRISTINE KONRADI, AND GEORGE M. ANDERSON

How are the approximately one hundred billion neurons in the central nervous system (CNS) arranged to process information? How do they communicate through their 1,000–10,000 connections with each other? Most importantly for psychiatrists and psychopharmacologists: How can environmental influences, such as a pharmacological intervention or psychotherapy, influence the way in which the brain processes information?

Two basic principles of neurotransmission help to understand normal brain function on the one hand and the current practice of clinical psychopharmacology on the other. First, the anatomic organization of neurotransmitter systems determines their input and output connections. Second, neurotransmitter receptors modulate the electrical properties (via ion channels) or the biochemical properties (via second-messenger systems) of neurons and, in turn, determine their activity. These two basic principles are reviewed here for the neurotransmitter systems considered most relevant to the practice of neuropsychopharmacology.

NEURAL CIRCUITRY

Neurons are arranged in distributed networks to govern human behavior (Mesulam 1998). This chapter focuses on four major anatomical systems: the cortex, the thalamus, the basal ganglia, and the medial temporal lobe (Fig. 2.1).

The thalamus is the gateway to cortical processing of all incoming sensory information, here represented by the three major systems: somatosensory, auditory, and visual (S, A, V) (Fig. 2.1). Primary sensory cortices (S1, A1, V1) receive information from the appropriate input modules (sensory organ + thalamus).

The association cortex integrates information from primary cortices, from subcortical structures, and from brain areas associated with memory, to create an internal representation of the sensory information.

The medial temporal lobe (i.e., hippocampus, amygdala) serves two major functions in the brain: to integrate multimodal sensory information for storage into and retrieval from memory, and to attach limbic valence to sensory information.

The basal ganglia are primarily involved in the integration of input from cortical areas. The basal ganglia modulate cortical activity via a cortico-striato-pallido-thalamo-cortical loop. The most prominent projections to the striatum arise from the motor cortex.

These four systems provide the anatomical basis for the three most basic brain functions: the reception of sensory information, the creation of an internal representation, and the creation of a response. Several other systems are involved in information processing in the brain (e.g., the cerebellum and the hypothalamus), but a focus on this core set of cerebral structures is warranted. The processing of information in this circuitry has to occur very quickly. For example, how else would the brain process a painful or threatening stimulus and produce an appropriate response to avoid the stimulus? All major pathways in this circuitry are glutamatergic, which, as we will see, allows for fast processing of information. The glutamatergic pathways are under inhibitory control within each of the brain regions by so-called interneurons, which use γ-amino butyric acid (GABA) as a neurotransmitter.

The function of these four systems is modulated by several groups of neurons that are characterized by their use of a specific neurotransmitter: cholinergic neurons in the basal forebrain and brainstem, dopaminergic neurons in the substantia nigra and ventral tegmental

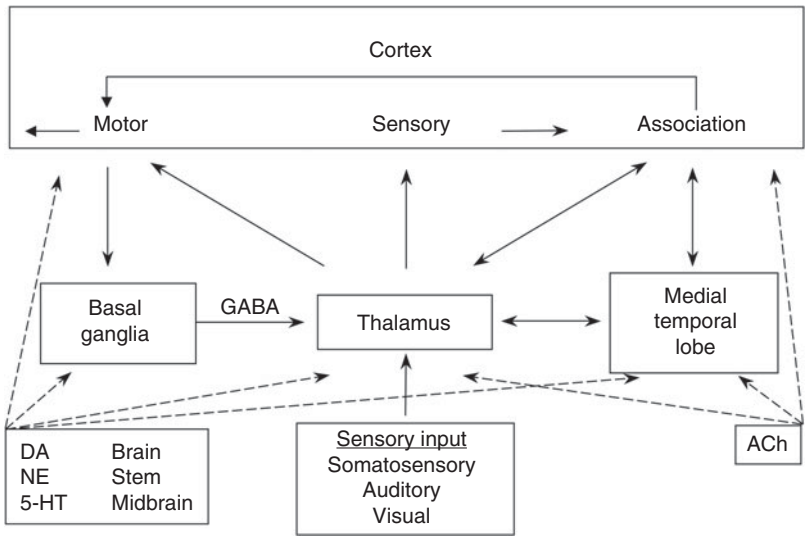

FIGURE 2.1 **Neural circuitry.** This diagram provides a basic scheme of information processing in the human brain. Excitatory neurons using glutamate as a neurotransmitter are shown in as arrows, inhibitory neurons using γ-aminobutyric acid (GABA) are shown as circles. The glutamatergic projection neurons are under inhibitory control by GABAergic interneurons. Diffuse-projecting neurotransmitter systems (using acetylcholine [ACh], dopamine [DA], norepinephrine [NE], and serotonin [5-HT]) located in the basal forebrain and brainstem are shown as dashed lines. Sensory information of the somatosensory, auditory, and visual realm arrive at the cortex via the thalamus. Primary sensory information is then relayed to association cortex, medial temporal lobe (MTL), and thalamus for further processing. One of the output modules, the motor cortex (M), is fine-tuned via inhibitory neurons in the basal ganglia (BG).

area, noradrenergic neurons in the locus ceruleus, and serotonergic neurons in the raphe nuclei. The broken arrows in Figure 2.1 indicate the four neurotransmitter-specific projection systems. Most of the therapeutic agents used in the current practice of psychopharmacology are aimed at strengthening or inhibiting these modulatory systems.

How do the four major anatomical systems and the neurotransmitter-specific diffuse projection systems communicate with each other? To answer this question, it is necessary to review the basic anatomy of a neuron.

Anatomy of the Neuron

Neurons have four major compartments: dendrites, cell bodies (perikaryon), axons, and terminals. Dendrites create a network of fibers providing the neuron with input from other cells. The cell body integrates the different inputs provided by the dendrites. This integration can occur through a modulation of the membrane potential, changes in second-messenger systems, or at the level of the nucleus (regulation of gene expression). A major function of the cell body is the synthesis of all cell-specific receptors and enzymes needed for neurotransmitter production.

The axon is the relay/output station of the neuron. The axon can be short (local circuit neuron) or long (projection neuron). If a deviation from the resting membrane potential is above a certain threshold, an action potential is created and travels downstream rapidly to terminal areas. Nerve terminals provide a small area of close contact with dendrites of neighboring cells: a *synapse*. Variations of this typical scheme include synapses between two axons, between two dendrites, and neurotransmitter release in medial parts of the axon (varicosities, boutons).

The Synapse

The terminal area of the presynaptic neuron releases the neurotransmitter into the synapse, and can express two types of proteins that affect synaptic communication (see 1 and 2 in Fig. 2.2):

- Membrane-bound receptors bind the intrinsic neurotransmitter (autoreceptor) or transmitters of neighboring neurons (heteroreceptor) and affect the cell via intracellular messengers. One response, for example, is the modulation of neurotransmitter release (Langer 1997).
- Membrane-bound reuptake transporters pump the released neurotransmitter back into the cell (Amara 1995; Lester et al. 1996).

The neuron receiving the input (postsynaptic cell) can be modulated via two different types of receptors (see 3 and 4 in Fig. 2.2):

- Fast-acting, class I (ionotropic) receptors: The neurotransmitter binds to the receptor protein and within milliseconds leads to a change in the permeability of the associated ion channel, allowing the influx of ions such as Ca^{++}, Na^{+}, K^{+} or Cl^{-}.

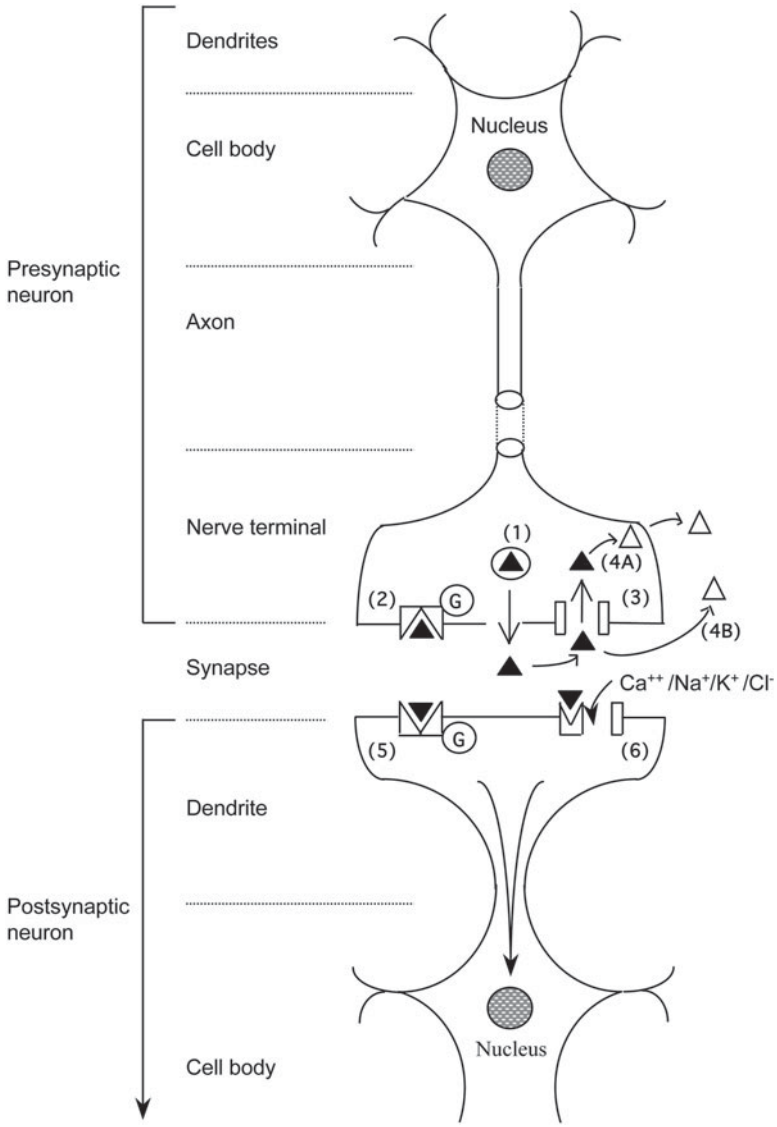

FIGURE 2.2 **The anatomy of the neuron.** Communication between two neurons occurs at the synapse. The presynaptic neuron produces and releases the neurotransmitter into the synaptic cleft. Four mechanisms (1–4) are important to understand the function of most neurotransmitter systems. The release of neurotransmitter can be modulated via presynaptic receptors (1). The amount of neurotransmitter in the synaptic cleft can be decreased by reuptake into the presynaptic neuron (2) or via enzymatic degradation. Neurotransmitter effects at the target neuron are relayed via fast acting ion-channel coupled receptors (3) or via slower acting G-protein-coupled receptors (4). Down-stream effects of postsynaptic receptors include the phosphorylation (P) of nuclear proteins.

- Slow-acting, class II (G-protein-coupled) receptors: The neurotransmitter binds to the receptor protein and thereby changes the protein conformation. This change is relayed to an associated *G-protein*, so called because it binds guanidine triphosphate (GTP) in order to be activated. G-proteins regulate two major classes of effector molecules: ion channels and second-messenger–generating enzymes.

Synaptic communication between neurons does not only involve the classical neurotransmitter systems reviewed in this chapter. Other major classes of neurotransmitters and neuromodulators are also known to affect brain function: these include the neuropeptides, fatty acid-based endocannabinoids (Piomelli 2005; Solinas et al. 2008), neuroactive steroids (Do Rego et al. 2009), and the gas transmitters such as nitric oxide and carbon monoxide. The neuropeptides (e.g., neurotensin, neuropeptide Y, substance P, CRH, and the opioid peptides) typically are synthesized in the cell body only and do not have active reuptake systems, being deactivated primarily by enzymatic degradation (Bodnar 2009; Hokfelt 1991). The gas neurotransmitters are not stored in vesicles, are not released by exocytosis, and also do not bind to postsynaptic receptors. Rather, they are freely diffusible and act on internal

receptors in their cell of origin and in neighboring cells (Mustafa et al. 2009; Snyder & Ferris 2000). We have chosen not to review these agents in detail, since their implications for the current practice of neuropsychopharmacology are limited. They do, however, offer promising targets for the development of new treatment strategies as discussed in the cited recent reviews.

NEUROTRANSMITTER SYSTEMS

Although there are a wide variety of neurotransmitter systems in the brain, clinical psychopharmacology is primarily concerned with six systems: the glutamatergic, GABAergic, cholinergic, serotonergic, noradrenergic, and dopaminergic. These six systems can be divided into two groups based on their anatomical characteristics.

The first group includes the glutamatergic and GABAergic systems. These two classes of neurons are by far the most prevalent and widely distributed neurotransmitter systems in the human brain. Thus, modulation of glutamatergic and GABAergic neurotransmission affects many neural systems.

The second group of neurotransmitter systems is composed of the previously mentioned modulatory systems, including the cholinergic, serotonergic, noradrenergic, and dopaminergic neurons. These four systems originate from small groups of neurons, densely packed in circumscribed areas of the forebrain or brainstem, which project to their target areas typically by long-ranging projection fibers. Since these neurotransmitter-specific projection systems typically reach selected neural systems, altering their function usually leads to more circumscribed effects.

Glutamatergic Neurotransmission

Glutamate (Glu) is the most abundant amino acid in the CNS, and about 30% of the Glu contained in the brain functions as the major excitatory neurotransmitter. It also an important intermediate in neuronal metabolism and is the immediate precursor for GABA, formed after decarboxylation of Glu by glutamic acid decarboxylase (GAD).

Anatomy Glutamatergic neurons are widely distributed throughout the entire brain. Most glutamatergic neurons are so-called *projection neurons*: their axon projects into distant brain regions. Prominent glutamatergic pathways are the connections between different regions of the cerebral cortex (cortico-cortical projections), the connections between thalamus and cortex, and the projections from cortex to striatum (extrapyramidal pathway) and from cortex to brainstem/spinal chord (pyramidal pathway).

The hippocampus is characterized by a series of glutamatergic neurons, which can create rhythms of electrical activity necessary for the generation of memory traces in the brain. The cerebellum, a region dedicated to the temporal processing of motor and cognitive information, is also rich with glutamatergic neurons (Ozawa et al. 1998).

Synaptic Organization Glutamate acts at three different types of ionotropic receptors (see 1 in Fig. 2.3) and at a family of G-protein-coupled (metabotropic) receptors (see 2 in Fig. 2.3) (Nakanishi 1992; Nakanishi et al. 1998; Vandenberg 1998).

Binding of glutamate to the ionotropic receptor opens an ion channel, allowing the influx of sodium ions (Na^+) and calcium ions (Ca^{2+}) into the cell. NMDA receptors bind glutamate and the eponymous *N*-methyl-d-aspartate. The receptor is comprised of two different subunits: $NMDAR_1$ (seven variants) and $NMDAR_2$ (four variants). The NMDA receptor is highly regulated at several sites. For example, the receptor is virtually ineffective unless a ligand (such as glycine) binds to the glycine site, and it can be blocked by the binding of ligands including MK-801, ketamine, and phencyclidine (PCP) to the PCP site inside the channel. AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors bind glutamate, AMPA, and quisqualic acid, whereas kainate receptors bind glutamate and kainic acid.

The metabotropic glutamate receptor family includes at least seven different types of G-protein-coupled receptors ($mGluR_{1-7}$). They are linked to different second-messenger systems and lead to the increase of intracellular Ca^{2+} or the decrease of cyclic adenosine monophosphate (cAMP). The increase of intracellular Ca^{2+} leads to the phosphorylation of target proteins in the cell.

Glutamate is removed from the synapse by high-affinity reuptake; two transporter proteins are expressed in glial cells and one in neurons (see 3 in Fig. 2.3). Once in the neuron, glutamate is transported into storage vesicles by vesicular glutamate transporters, which serve as excellent markers of gluatmatergic neurons (Takamori 2006).

Function The widespread distribution of glutamatergic neurons explains why glutamate is involved in many brain functions. Modulation of glutamatergic activity is, therefore, most likely to have widespread effects. Excess stimulation of glutamatergic receptors, as seen in seizures or stroke, can lead to unregulated Ca^{2+} influx and neuronal damage and cytotoxicity (Coyle & Puttfarcken 1993; Dingledine et al. 1990; Loscher 1998).

Several brain functions have been linked to specific glutamate receptor subtypes in selected brain regions.

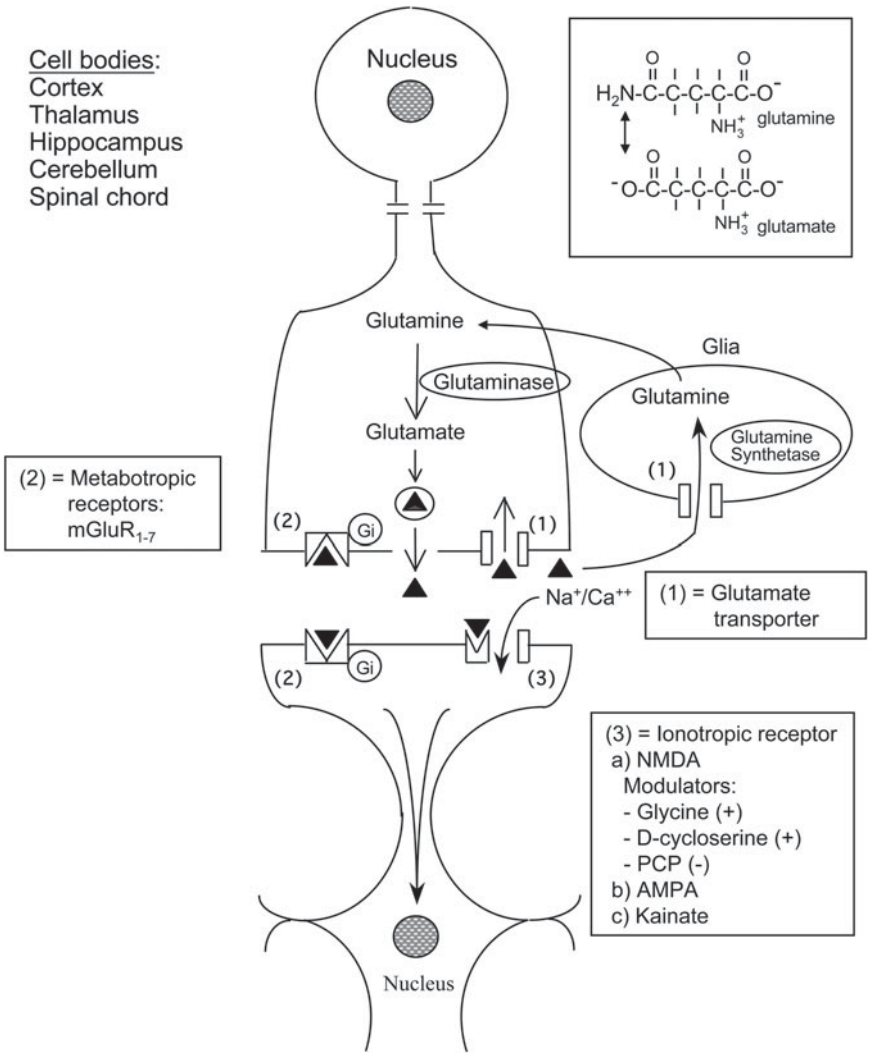

FIGURE 2.3 **Glutamatergic synapse.** Glutamate binds to ionotropic receptors (1) and metabotropic receptors (2). The membrane glutamate transporter (3) pumps glutamate back into the glutamatergic neuron, and also into neighboring glia, where it is converted to glutamine and returned to the neuron.

For example, glutamatergic neurons and NMDA receptors in the hippocampus are important for long-term potentiation (LTP), a crucial component in the formation of memory (Wilson & Tonegawa 1997). Animal models with selective lesioning or strengthening of NMDA receptors in the hippocampus have demonstrated that this glutamate receptor subtype is crucial for normal memory function.

Abnormalities of the glutamate system have also been implicated in neuropsychiatric disorders. For example, compounds such as PCP and ketamine, which block the NMDA receptor, can induce psychotic symptoms. On the other hand, compounds such as d-cycloserine or glycine, which increase NMDA receptor function via the glycine binding site, have been reported to decrease psychotic and/or negative symptoms in schizophrenia (Farber et al., 1999, Goff et al., 1999; Heresco-Levy et al., 1999; Labrie & Roder 2010; Millan, 2005; Tsai & Lin, 2009). However, the clinical utility of agents active at ionotropic GluRs to increase or inhibit excitatory transmission in pathological states remains to be determined. Concerted efforts are being made to develop metabotropic Glu receptor agents for a range of conditions (Recasens et al. 2008), including epilepsy (Tang et al. 2009), schizophrenia (Conn et al. 2009), anxiety and depression (Palucha & Pilc 2008), and Fragile X syndrome (Dolen & Bear 2008).

GABAergic Neurotransmission

γ-Aminobutyric acid (GABA) is an amino acid found in high concentrations in the brain and the spinal chord, where it is produced from Glu by the action of GAD (existing as two major forms GAD_{65} and GAD_{67}).

GABA acts as the major inhibitory neurotransmitter in the CNS and is an excellent marker for GABAergic neurons, with little found elsewhere.

Anatomy GABAergic neurons can be divided into two groups, short-ranging neurons that connect to other neurons in the same brain region and medium-/long-ranging neurons that project to distant brain regions. The vast majority of GABAergic neurons are short-ranging neurons (also called *interneurons* or *local circuit neurons*) in the cortex, thalamus, striatum, cerebellum, and spinal cord. Various subtypes of the GABAergic interneurons provide tonic as well as phasic inhibitory control over glutamatergic projection cells. An intricate balance of inhibitory (GABAergic) and excitatory (glutamatergic) tone is essential for normal function (Somogyi et al. 1998).

Three groups of medium-/long-ranging GABAergic neurons have projections into other brain regions. The most important group consists of projections from the caudate/putamen to the globus pallidus, and from the globus pallidus to the subthalamic nucleus and to the thalamus. These GABAergic projections in the striato-pallido-thalamic pathway function as part of a larger cortico-striato-pallido-thalamo-cortical circuit involved in modulating cortical output. Another important group of GABAergic projection neurons connect the septum with the hippocampus and is important in several hippocampal functions, including memory. The third group of long-ranging GABAergic neurons projects from the substantia nigra to thalamus and superior colliculus.

Synaptic Organization GABA acts at two types of receptors (see 1 and 2 in Fig. 2.4). The $GABA_A$ receptor is a receptor–channel complex comprised of five subunits (Lüddens & Korpi 1996). Activation leads to the opening of the channel, allowing Cl^- to enter the cell, resulting in decreased excitability. Five distinct classes of subunits (six variants of α, four variants of β, three variants of γ, one δ, and two variants of ρ) are known. Multiple variations in the composition of the $GABA_A$ receptor are known, but the prominent type is created by two α, two β, and one γ or δ subunit. The receptor can be modulated by various compounds that bind to several different sites:

- Agonists like GABA, muscimol, and progabide bind between the γ and β subunits and open the chloride channel.
    - Benzodiazepines bind to the α subunit and open the channel if a γ subunit is present and if GABA is bound to the GABA site on the β subunit.
    - Barbiturates and ethanol bind near the Cl^- channel and increase channel open time even without GABA present.

The $GABA_B$ receptor is a G-protein-coupled receptor with similarity to the metabotropic glutamate receptor (Bettler et al. 1998; Kaupmann et al. 1997). The $GABA_B$ receptor is linked to G_i (decreasing cyclic AMP and opening of K^+ channels) and G_o (closing Ca^{2+} channels). The net effect is prolonged inhibition of the cell. A well-known agonist is baclofen. The $GABA_B$ receptor is found postsynaptically (causing decreased excitability) and presynaptically (leading to decreased neurotransmitter release).

GABA is removed from the synapse by sodium-dependent GABA uptake transporters (3 in Fig. 2.4), of which there are several forms.

Function γ-Aminobutyric acid is the major inhibitory neurotransmitter in the CNS. Similar to the modulation of glutamatergic receptors, the application of agents that can strengthen or weaken GABAergic activity has widespread effects.

The cortical, hippocampal, and thalamic GABAergic neurons are crucial for the inhibition of excitatory neurons. Foci of local imbalance, with a subnormal tone of GABAergic inhibition, may spread to distant areas to induce a seizure. $GABA_A$ agonists such as benzodiazepines or barbiturates can decrease the occurrence of seizures or interrupt ongoing seizure activity (Bazil & Pedley 1998).

Modulation of $GABA_A$ receptors is also beneficial in the treatment of several neuropsychiatric conditions, including anxiety disorders, insomnia, and agitation. The mechanisms are not well understood, but may work through a general inhibition of neuronal activity. Benzodiazepines and ethanol both enhance $GABA_A$ receptor function, and this common property is the basis for ethanol detoxification with benzodiazepines (Grobin et al. 1998).

Finally, psychotic disorders, especially schizophrenia and bipolar disorder, have been associated with abnormalities of specific subtypes of GABAergic neurons, primarily in the prefrontal cortex and the paralimbic cortex (anterior cingulate gyrus and hippocampus) (Benes & Berretta 2001; Gaspar et al. 2009; Lewis et al. 1999; Lewis & Hashimoto 2007).

Cholinergic Neurotransmission

Acetylcholine (ACh) has been known as a neurotransmitter since the mid 1920s. In fact, the demonstration that acetylcholine is the *Vagusstoff* ("vagus-substance") released from the vagus nerve to modulate heart function was the first proof for the chemical mediation of nerve impulses (Loewi & Navratil 1926). In the peripheral nervous system, acetylcholine is found as the neurotransmitter in the autonomic ganglia, the parasympathetic postganglionic synapse, and the neuromuscular endplate. Centrally and peripherally,

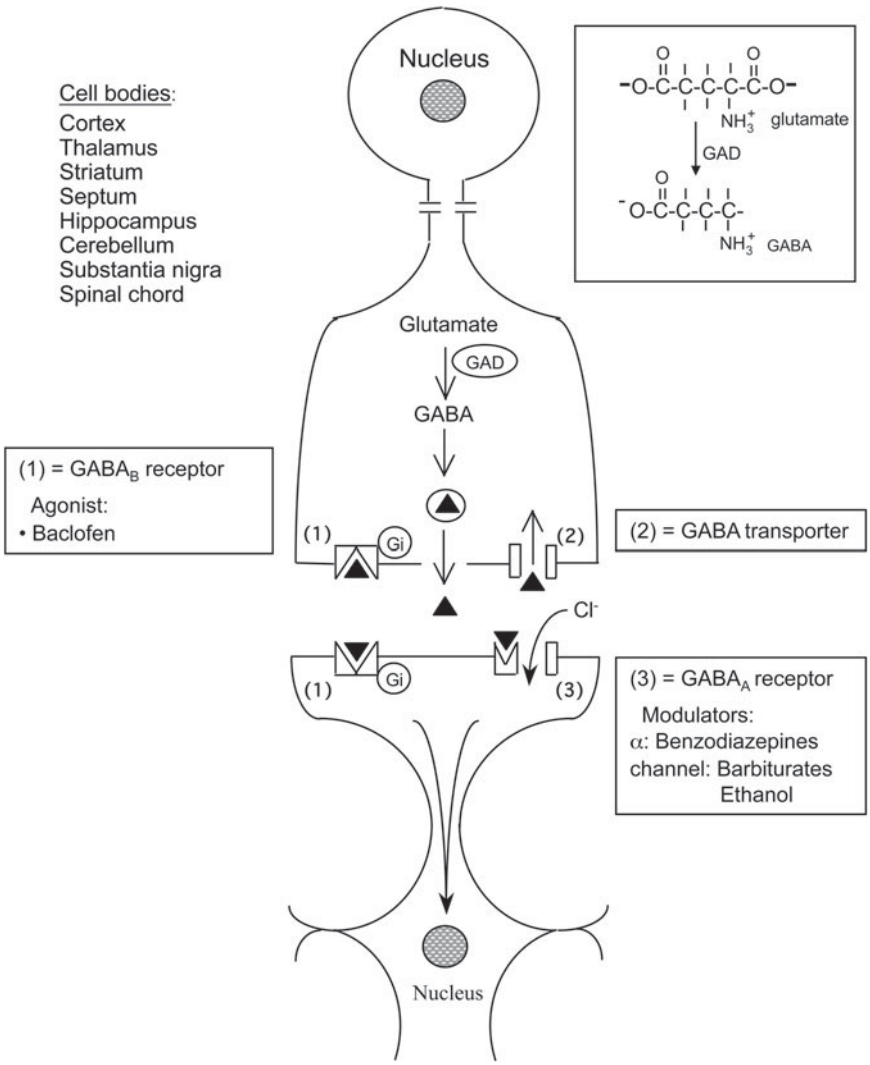

FIGURE 2.4 **GABAergic synapse.** γ-Aminobutyric acid (GABA) binds to $GABA_A$ receptors (1) and $GABA_B$ receptors (2). The GABA transporter pumps GABA back into the GABAergic neuron.

acetylcholine is produced from the precursors acetyl-CoA and choline through the action of the enzyme choline acetyltransferase.

Anatomy Cholinergic neurons in the CNS are either wide-ranging projection neurons or short-ranging interneurons. The most prominent group of cholinergic neurons is found in the basal forebrain and include subgroups in the septum, diagonal band, and nucleus basalis of Meynert. These cholinergic basal forebrain neurons project to many areas of the cerebral cortex, but with regional specificity. The projections are weak in primary sensory and motor areas, become more prominent in association cortex, and are most prominent in paralimbic cortical areas (e.g., cingulate gyrus and hippocampus) (Mesulam 1996). A smaller group of cholinergic projection neurons are located in the brainstem and project predominantly to the thalamus. The only group of short-ranging cholinergic interneurons are in the striatum and modulate the activity of GABAergic striatal neurons.

Synaptic Organization Acetylcholine acts at two different types of cholinergic receptors (see 1 and 2 in Fig. 2.5). Muscarinic receptors bind ACh, as well as other agonists (muscarine, pilocarpine, bethanechol) and antagonists (atropine, scopolamine). There are at least five different types of muscarinic receptors (M_1–M_5). All have slow response times. They are coupled to G-proteins and a variety of second-messenger systems. When activated, the final effect can be to open or close channels for K^+, Ca^{2+}, or Cl^- (Bonner 1989).

Nicotinic receptors are less abundant than the muscarinic type in the CNS. They bind ACh, as well

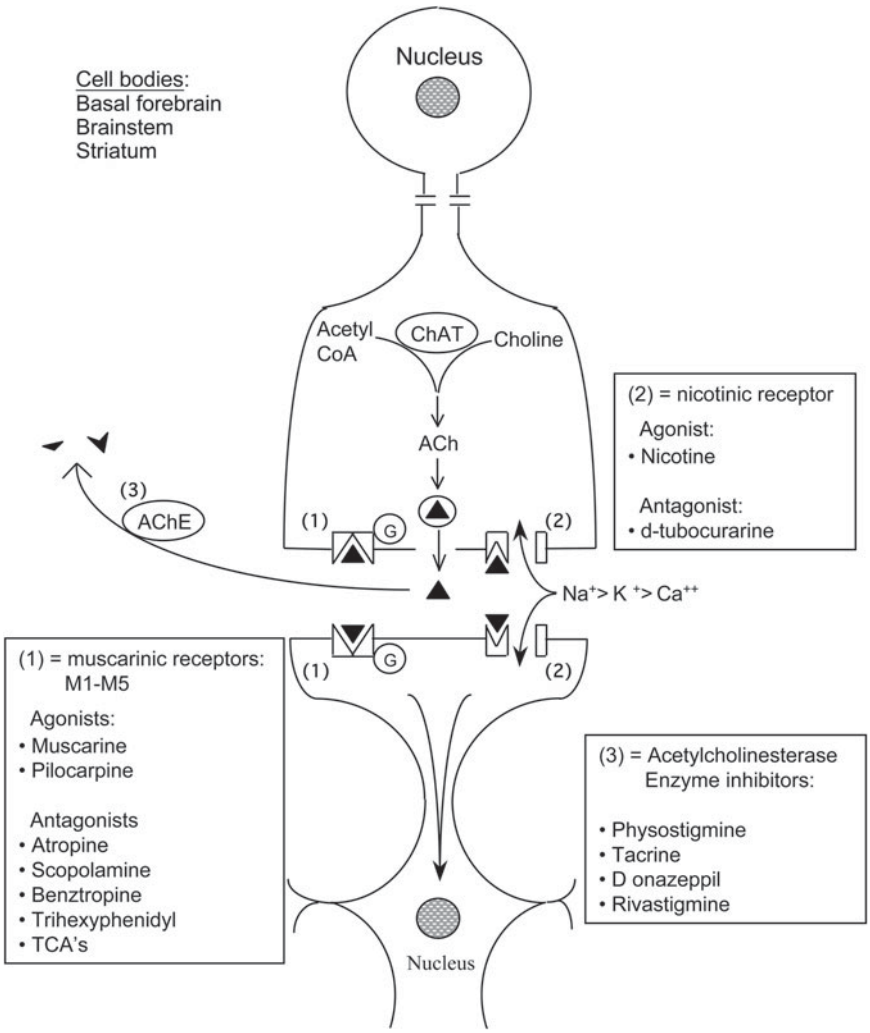

FIGURE 2.5 **Cholinergic synapse.** Acetylcholine binds to muscarinic receptors (1) and nicotinic receptors (2). Acetylcholinesterase (AChE) cleaves the neurotransmitter acetylcholine into choline and acetate.

as agonists such as nicotine or antagonists such as d-tubocurarine. The fast-acting, ionotropic nicotinic receptor allows influx of $Na^+ > K^+ > Ca^{2+}$ into the cell.

Presynaptic cholinergic receptors are of the muscarinic or nicotinic type and can modulate the release of several neurotransmitters (Wonnacott 1997). Acetylcholine is removed from the synapse through hydrolysis to acetate and choline by the enzyme acetyl cholinesterase (AChE). Removal of ACh from the synapse can be effectively blocked by inhibitors of AChE—irreversibly by organophosphorous compounds and in a reversible fashion by drugs such as physostigmine.

Function The projection patterns of the cholinergic neurons help to explain their behavioral associations. Cholinergic neurons in the nucleus basalis of Meynert provide tonic and phasic activation of the cerebral cortex to modulate attention and novelty seeking (Detari et al. 1999). The cholinergic neurons of the septum and diagonal band projecting to the hippocampus are essential for normal memory function (Baxter & Chiba 1999). Alzheimer disease (AD) and anticholinergic delirium are two examples of a cholinergic deficit state. Blocking the metabolism of ACh through inhibition of AChE can slightly improve cognitive functioning or slow cognitive decline in AD patients and reverses the acute confusional state induced by anticholinergic drugs (Geula 1998; Giacobini 1998).

The brainstem cholinergic neurons are essential for the regulation of sleep–wake cycles via projections to the thalamus. The cholinergic interneurons in the striatum modulate striatal GABAergic neurons by opposing the effects of dopamine. Increased cholinergic tone in Parkinson disease and cholinergic side effects such as sialorrhea in patients treated with certain neuroleptics appear to arise from decreased dopaminergic inhibition of cholinergic neurons in the striatum (Calabresi et al. 2000).

Serotonergic Neurotransmission

In the periphery, the indoleamine serotonin or 5-hydroxytryptamine (5-HT) is found principally in the gut wall, where it plays a critical role in peristalsis, and in the platelet, where it is important in hemostasis. Although only 1%–2% of the entire body content of 5-HT is found in the CNS, brain, and spinal cord 5-HT is crucial to a wide range of behaviors and cognitive processes. Serotonin is produced from the essential amino acid tryptophan after hydroxylation and decarboxylation. The rate-limiting hydroxylation step is catalyzed by tryptophan hydroxylase, of which there are two forms: TPH1, found mainly in the periphery, and TPH2, expressed exclusively in neurons.

Anatomy Cell bodies of central serotonergic neurons are found only in midline structures of the brainstem. Most serotonergic cells overlap with the distribution of the raphe nuclei in the brainstem. A rostral group (B6–8) projects to the thalamus, hypothalamus, amygdala, striatum, and cortex. The remaining two groups (B1–5) project to other brainstem neurons, the cerebellum, and the spinal cord.

Synaptic Organization Serotonin acts at two different types of receptors: G-protein-coupled receptors (see 1 and 2 in Fig. 2.6) and an ion-gated channel (see 3 in Fig. 2.6) (Julius 1991). With the exception of the 5-HT_3 receptor, all serotonin receptors are G-protein-coupled and can be grouped as follows:

1. The 5-HT_1 receptors (5-$HT_{1A, 1B/D, 1C, 1E, 1F}$) are coupled to G_i and lead to a decrease of cyclic AMP. The 5-HT_{1A} receptor is also directly coupled to a K^+ channel, leading to increased opening of the channel. The 5-HT_{1A} and 5-HT_{1B} receptors are the predominant serotonergic autoreceptors.
2. 5-HT_2 receptors (5-HT_{2A} and 5-HT_{2C}) are coupled to phospholipase C and lead to a variety of intracellular effects (mainly depolarization). Three receptors (5-HT_4, 5-HT_6, and 5-HT_4) are coupled to G_s and activate adenylate cyclase. The function of the 5-HT_{5A} and 5-HT_{5B} receptors is poorly understood, although localization and functional studies suggest a role for the 5-HT_{5A} receptor in circadian rhythms, mood, cognition and perception (Thomas 2006).
3. The 5-HT_3 receptor is the only monoamine receptor coupled to an ion channel, probably a Ca^{2+} channel. It is found in the cortex, hippocampus, and area postrema. It is typically localized presynaptically and regulates neurotransmitter release. Well-known antagonists are the potent antiemetics ondansetron and granisetron.

Serotonin is removed from the synapse by a high-affinity serotonin uptake site (4 in Fig. 2.6) that is capable of transporting serotonin in either direction, depending on its concentration. The serotonin transporter is blocked by the selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and sertraline, as well as by tricyclic antidepressants. Serotonin is metabolized intraneuronally to 5-hydroxyindoleacetic acid by monoamine oxidase-A (MAO-A).

Function Serotonin has been linked to many brain functions and is suggested to be involved in many psychiatric symptoms, which is not surprising considering the widespread serotonergic projections and the heterogeneity of the serotonergic receptors (Jacobs & Azmitia 1992; Lucki 1998).

Increasing serotonergic tone through inhibition of the serotonin transporter has proved useful in the treatment of anxiety, depression, and obsessive-compulsive disorder (Murphy et al. 1998). The 5-HT_{1A} partial agonist buspirone has anxiolytic effects. Many of the newer atypical antipsychotic agents exert their effects at least partially through blockade of 5-HT_2 receptors (Meltzer 1999). Conversely, the hallucinogens LSD and psilocybin, although having affinity for a wide range of serotonin receptors, appear to exert their effects principally by acting as partial agonists at 5-HT_{2A} receptors located on apical dendrites of cortical pyramidal neurons (Aghajanian & Marek 1999; Geyer & Vollenweider 2008). The antimigraine drug sumatriptan and related triptans are agonists at the 5-HT_{1D} receptor.

Dopaminergic Neurotransmission

The catecholamine dopamine (DA) is formed from the amino acid tyrosine, after tyrosine hydroxylase (TH) catalyzed hydroxylation at the 3 position of the phenyl ring of tyrosine, followed by decarboxylation. Dopamine was initially considered to be merely an intermediate in the synthesis of norepinephrine and epinephrine; however, in the late 1950s, DA was discovered to be an important neurotransmitter in its own right.

Anatomy There are three groups of dopaminergic neurons in the human, which differ in the length of their efferent fiber systems.

Wide-ranging projections are especially well studied, having their cell bodies in two neighboring regions of the brainstem, substantia nigra (SN), and ventral tegmental area (VTA). The SN neurons, also called A9 neurons, project primarily to the caudate and putamen. The VTA neurons, also called A10 neurons, project to limbic areas, such as the nucleus accumbens and amygdala (so-called mesolimbic projections), and to the frontal, cingulate, and entorhinal cortex (so-called mesocortical projections).

Intermediate-length systems originate in the hypothalamus, and a principle pathway (the tuberoinfundibular)

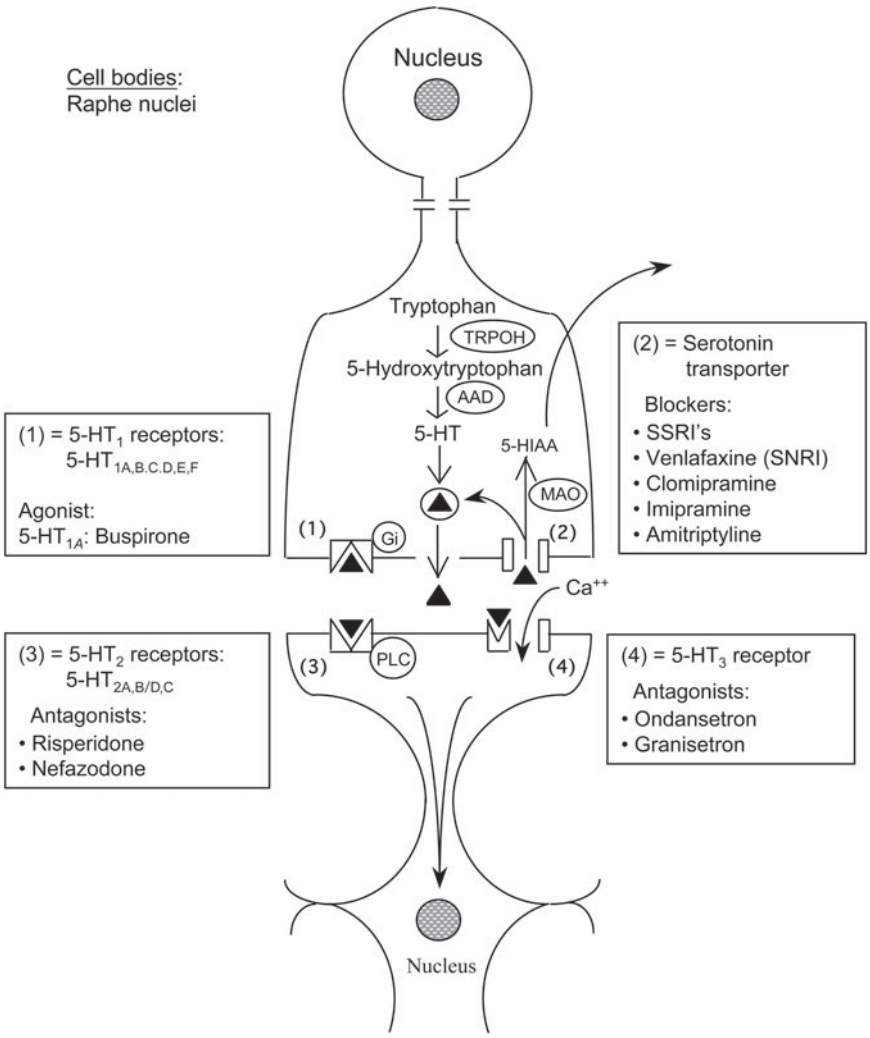

FIGURE 2.6 **Serotonergic synapse.** Serotonin binds to approximately 15 different receptors. The most relevant in psychopharmacology are the 5-HT_1 receptors (1), the 5-HT_2 receptors (3), and 5-HT_3 receptors (4). Antagonists of the 5-HT_2 receptor include ketanserin and most of the atypical antipsychotic drugs. The serotonin transporter (2) pumps serotonin back into the neuron and can be blocked by tricyclic antidepressants and more selectively by the selective serotonin reuptake inhibitors (SSRIs) including fluoxetine, citalopram, and sertraline. The vesicle is depicted in the center of the terminal area; not shown is the vesicular monoamine transporter (VMAT), which transports serotonin, and the other monoamines including dopamine and norepinephrine into vesicles.

projects to the pituitary gland and is important in regulation the secretion of prolactin. Finally, ultra-short systems are found in the retina and olfactory bulb.

Synaptic Organization Dopamine is released into the synapse from vesicles (see 1 in Fig. 2.7); this process is facilitated by the stimulants amphetamine and methylphenidate. Dopamine acts at two different classes of dopamine receptors in the CNS, the D_1 receptor family (see 2 in Fig. 2.7) and the D_2 receptor family (see 3 in Fig. 2.7) (Baldessarini & Tarazi 1996). The D_1 receptor family includes the D_1 and D_5 receptors. Both are coupled to G_s and lead to an increase of cyclic AMP. The D_2 receptor family includes the D_2, D_3, and D_4 receptors. All are coupled to G_i and lead to a decrease of cAMP. There is a predilection of the different dopamine receptors for expression in specific brain areas: D_1, striatum, cortex, SN, olfactory tubercle; D_2, striatum, SN, pituitary gland, retina, olfactory tubercle; D_3, nucleus accumbens; D_4, on GABAergic neurons in cortex, thalamus, hippocampus, SN; D_5, hippocampus, cortex, hypothalamus.

Presynaptic dopaminergic receptors are typically of the D_2 type and found on most portions of the dopaminergic neuron (as autoreceptors). They regulate DA synthesis and release, as well as the firing rate of DA neurons. Autoreceptors are five to ten times more sensitive to DA agonists than are postsynaptic receptors.

Dopamine is removed from the synapse by two mechanisms: catechol-O-methyl-transferase (COMT) degrades intrasynaptic DA, while the dopamine transporter (DAT) (see 4 in Fig. 2.7), a Na^+/Cl^- dependent

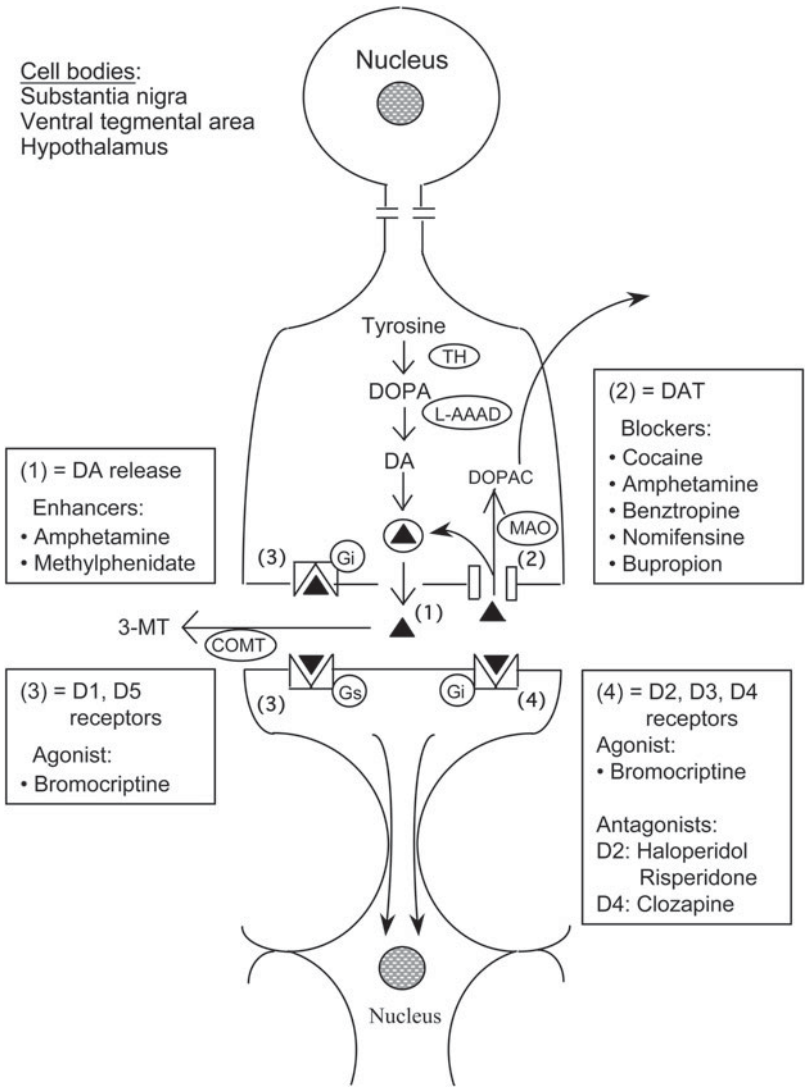

FIGURE 2.7 **Dopaminergic synapse.** The release of dopamine (1) can be enhanced and stimulated by compounds such as amphetamine and methylphenidate. Once released, dopamine binds to two types of dopamine receptors. The family of D_1 receptors includes the D_1 and D_5 receptors (2), the family of D_2 receptors includes the D_2, D_3, and D_4 receptors (3). Dopamine is removed from the synapse predominantly via uptake by the dopamine transporter (4), and also through catabolism (methylation) by catechol-O-methyltransferase (COMT).

neurotransmitter transporter, transports DA back into the neuron. The DAT is blocked selectively by drugs such as cocaine, amphetamine, bupropion, and nomifensine. In humans, most dopamine is first metabolized intraneuronally by the monoamine oxidase (type B).

Function As with most neurtransmitter systems, the effects of dopamine on brain function are inextricably linked to its projection patterns. Furthermore, dopamine affects brain functions primarily by modulation of other neurotransmitter systems (Missale et al. 1998). The dopaminergic projections of the SN to the striatum modulate the neuronal excitability of GABAergic neurons in the striatum (Joel & Weiner 2000; Nicola et al. 2000). By targeting either D_1 or D_2 receptors on different subpopulations of GABAergic projection neurons, they are in the position to specifically alter the flow of information within the cortico-striato-pallido-thalamocortical circuit subserving several behaviors, including motor function and cognition. Parkinson disease and extrapyramidal side effects due to treatment with neuroleptics are examples of a decreased function of this dopaminergic projection.

Dopaminergic projections of the VTA to limbic structures, such as the nucleus accumbens, are known to be involved in appetitive and reward behavior and in the development of addiction to drugs such as cocaine, nicotine, and opiates (Berke & Hyman 2000; Diana 1998; Koob 1998; Spanagel & Weiss 1999). Dopaminergic projections of the VTA to the cortex play a role in the fine-tuning of cortical neurons (i.e., improving signal-to-noise

ratio) (Goldman-Rakic 1998). It appears that dopaminergic projections to pyramidal neurons act via D_1 receptors, whereas dopaminergic projections to GABAergic interneurons act via D_4 receptors (Goldman-Rakic et al. 2000). This could provide the basis for developing agents with specific effects on dopaminergic modulation of cortical function, based on their preference for postsynaptic dopaminergic receptors.

Dopaminergic projections from the hypothalamus to the pituitary gland tonically inhibit the production and release of prolactin via D_2 receptors. The blockade of these receptors leads to hyperprolactinemia, a common side effect of typical and atypical antipsychotic drugs.

Noradrenergic Neurotransmission

Norepinephrine (NE), a catecholamine like dopamine, was first identified as a neurotransmitter in 1946. In the peripheral nervous system, it is found as the neurotransmitter at the sympathetic postganglionic synapse. Peripherally and centrally, NE is produced through the action of dopamine-β-hydroxylase (DBH) on dopamine; NE also serves as the immediate precursor for epinephrine (adrenaline).

Anatomy The cell bodies of about half of all central noradrenergic neurons are located in the locus ceruleus, where approximately 12,000 reside on each side of the brainstem. They provide the extensive noradrenergic innervation of cortex, hippocampus, thalamus, cerebellum, and spinal cord. The remaining neurons are distributed in the tegmental region and innervate predominantly the hypothalamus, basal forebrain and spinal cord.

Synaptic Organization Norepinephrine is released into the synapse from vesicles (1 in Fig. 2.8); stimulants such as amphetamine and methylphenidate facilitate this release. Norepinephrine acts in the CNS by stimulating two major classes of adrenergic receptors, the α and β (see 2 and 3 in Fig. 2.8). Adrenergic α receptors can be subdivided into α_1 receptors (coupled to phospholipase and located postsynaptically) and α_2 receptors (coupled to G_i and located primarily presynaptically) (Insel 1996).

Adrenergic β receptors in the CNS are predominantly of the β_1 subtype (3 in Fig. 2.8). β_1 receptors are coupled to G_s and lead to an increase of cAMP. Cyclic AMP triggers a variety of events mediated by protein kinases, including phosphorylation of the β receptor itself, and regulation of gene expression via phosphorylation of transcription factors.

Norepinephrine is removed from the synapse by two mechanisms: (1) COMT degrades intrasynaptic NE, (2) the norepinephrine transporter (NET), a Na^+/Cl^--dependent neurotransmitter transporter, is the primary route of removal of NE from the synapse (4 in Fig. 2.8). The NET is blocked selectively by desipramine and nortriptyline. Once internalized, NE is either repackaged in synaptic vesicles after uptake by the vesicular monoamine transporter (VMAT) or degraded by the intracellular enzyme MAO-A. It should be noted that, under most circumstances, most of the NE synthesized in the neuron is never released, but metabolized intraneuronally. Although appearing inefficient, this excess synthesis actually provides a ready reservoir when demand for releasable NE increases (Eisenhofer et al. 2004).

Function By targeting the thalamus, limbic structures, and cortex, noradrenergic projections are important in arousal, orientation to novel stimuli, selective attention, and vigilance. Noradrenergic input is also involved in the modulation of sleep cycles, appetite, mood, and cognition.

An important anatomical feature of the locus ceruleus (LC) is the rich innervation by afferents from the sensory systems. This puts the LC in the position to monitor the internal and external environments. The widespread LC efferents in turn then lead to an inhibition of spontaneous discharge in the target neurons. Therefore, the LC is thought to be crucial for fine-tuning the attentional matrix of the cortex and the activity in limbic structures. Perturbations of this system may contribute to anxiety disorders.

The LC neurons express somatodendritic (cell body) α_2 autoreceptors, which allows α_2 receptors agonists such as clonidine and guanfacine to decrease LC firing and α_2 antagonists such as yohimbine to increase LC firing (Buccafusco 1992). Clonidine is used in the treatment of opiate withdrawal, Tourette syndrome, and post-traumatic stress disorder (PTSD), conditions in which it is considered desirable to decrease noradrenergic functioning (Southwick et al. 1999). An increase of noradrenergic function appears to convey some of the therapeutic effect of antidepressants (Anand & Charney 2000; Charney 1998; Frazer 2000), and selective stimulation of cortical postsynaptic α_2 receptors has been shown to improve cognitive functioning (Arnsten et al. 1996).

CONCLUSION

Information processing in the human brain via neurochemically defined neuronal systems is complex. Therefore, it remains a challenge to conceptualize psychiatric disorders and their treatment in a reductionistic framework of chemical neuroanatomy. Here, we have demonstrated that the anatomic organization of neurotransmitter systems determines their behavioral and cognitive effects, and that receptors modulate the electrical or biochemical properties of neurons, with direct

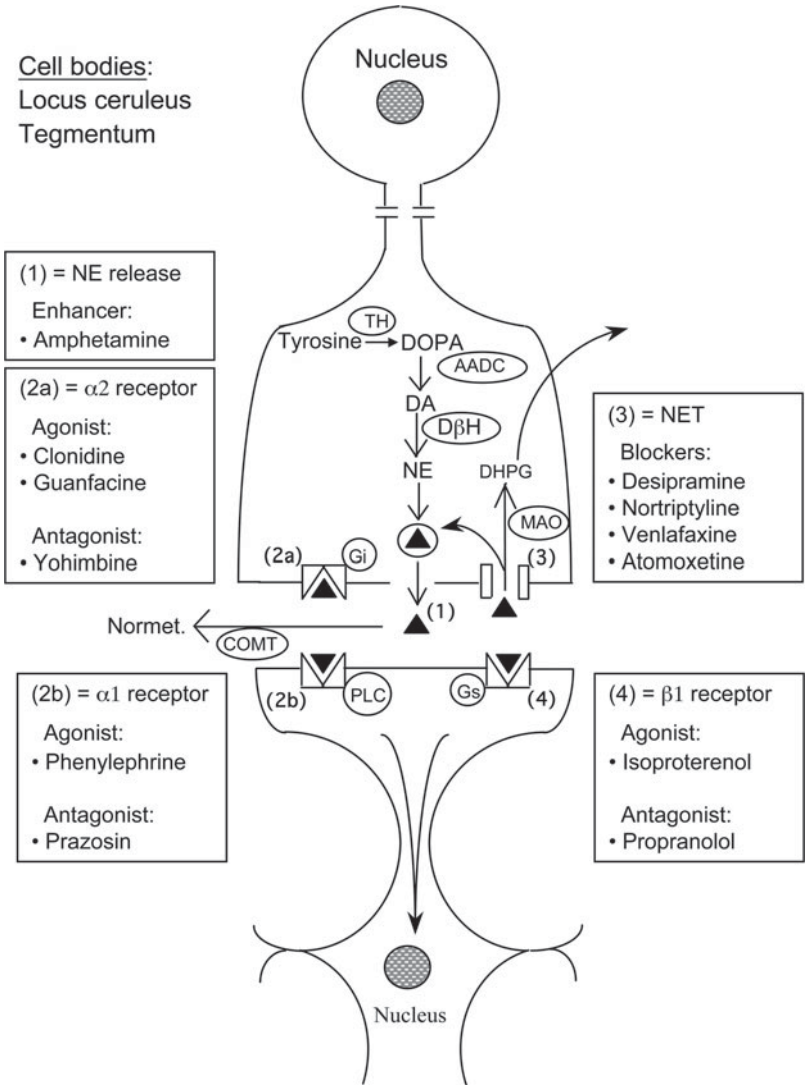

FIGURE 2.8 **Noradrenergic synapse.** The release of norepinephrine (1) can be enhanced by compounds such as amphetamine. Once released, norepinephrine binds to α_2 receptors (2a), α_1 receptors (2b), and β_1 receptors (3). Norepinephrine is removed from the synapse predominantly via reuptake by the norepinephrine transporter, and also through catabolism (methylation) by catechol-O-methyltransferase (COMT).

relevance to the mechanism of action of psychotropic drugs. Future research will provide more knowledge regarding the underlying neurobiology and the subtypes of neurons and neurotransmitters systems involved in the regulation of normal and abnormal behavior and cognition. This should lead to an increasingly rational approach to the development of new drug targets and treatment interventions.

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3

Developmental Principles of Pharmacokinetics

ALEXANDER A. VINKS, SHANNON N. SALDAÑA, AND PHILIP D. WALSON

Rational pharmacotherapy is dependent upon a basic understanding of the way patients handle drugs (pharmacokinetics) and their response to specific drug effects (pharmacodynamics). Most drugs have been developed and tested only in young to middle-aged adults. The evaluation of medications in children has always lagged behind that in adults. In 1964, Dr. Harry Shirkey first called attention to this major public health problem. He coined the term "therapeutic orphans" to describe children, as a result of the lack of formal drug studies in children (Shirkey 1968, 1999).

Important advances in pediatric clinical pharmacology have been made since then, although reluctance to pursue pharmacokinetics and pharmacodynamic studies in children remain (Kauffman & Kearns 1992). Consequently, even at present, many drugs that are being routinely used in children have not been adequately tested for safety and efficacy in this population (Banner 2002; Blumer 1999; Jong et al. 2000; Volkers et al. 2007). The primary reasons for this situation are the ethical constraints fueled by fears and anxiety about doing studies in children and the fact that drug companies have had little economic incentive to study drugs in the pediatric population. Any use of a drug in children not described in the U.S. Food and Drug Administration (FDA)-approved labeling is considered "off-label." Because the label frequently does not contain pediatric dosing information, doses are often derived from the adult dose, adjusted for body weight or body surface area. This, however, does not take into account all aspects of pediatric physiology, and can thus put the child or adolescent at risk for failing therapy or for adverse drug reactions due to inappropriate dosing.

In 1990, the Institute of Medicine sponsored a workshop to address the lack of pediatric labeling. This workshop produced recommendations that eventually led to the pediatric provisions of the FDA Modernization Act of 1997, as well as to the 1998 FDA Pediatric Rule (FDAMA) (1999). These eventually led to the Best Pharmaceuticals for Children Act (BPCA) and the Pediatric Research Equity Act (PREA) (FDA 2009). These acts in the United States have been followed by similar legislation in Europe, as evidenced by The National Institute for Health Research Medicines for Children Research Network from the United Kingdom (Abdurrahman et al. 2007; Filler et al. 2005; SENSE 2009), and the Pediatric Regulation from the European Medicines Agency in act since January 2007 (EMEA 2009). Stimulated by these acts, many more protocols now include early studies in children with emphasis on pharmacokinetics and pharmacodynamic behaviors that are important to understand and predict developmental differences in drug response.

THE TARGET CONCENTRATION INTERVENTION (TCI) STRATEGY

Although imperfect, there is almost always a better relationship between the action of a given drug and its concentration in the blood or at its site(s) of effect than between the amount in milligrams or the milligram per kilogram dose of the drug given and the observed effect. Pharmacokinetics is the science that can explain and predict the relationship between a dosing regimen and the concentration of a drug in various body compartments of individuals or groups of individuals. Pharmacokinetics may be simply defined as what the body does to the drug, as opposed to pharmacodynamics, which may be defined as what the drug does to the body (Benet 1984). A basic understanding of pharmacokinetic principles and the effects of development on them are required to better understand and predict drug actions, as well as to interpret drug concentration measurements.

In Figure 3.1 the interrelationship among drug input (dose), adherence, pharmacokinetics, pharmacodynamics (receptor interaction and [patho]physiology), and clinical effects is schematically conceptualized (Kenna et al. 2005).

Many practical, physiologic, and pathophysiologic factors determine how much drug effect will be associated with a drug prescription. Clinicians make a diagnosis and then prescribe a dosing regimen: drug, dose, formulation, route, frequency, and duration. Once a drug is prescribed, many factors determine how much effect, either therapeutic or toxic, is seen in the individual patient. Prescriptions must be filled correctly (the prescription filled must contain the correct drug and amount), and the dosage regimen must be taken (adherence; the drug must get into the patient and get to the site[s] of action). There are many reasons why concentrations and drug exposure (and therefore the effects) that result from prescriptions differ among patients. Even if taken or given exactly as desired, effects produced will depend on many factors including the patient's physiology, prior or current drug therapy, and other drugs present. Patients or parents may never fill the prescription. Up to 25% of patients do not fill prescriptions, and even more do not take medication as indicated. Children and adolescents with chronic illness have great difficulty completing prescribed treatment regimens, which can be complex and burdensome. High rates of nonadherence to treatment (up to 50% or more) have been reported for various pediatric chronic conditions, such as asthma, epilepsy, transplantation, juvenile rheumatoid arthritis (JRA), and diabetes (Kahana et al. 2008; Modi et al. 2008; Quittner et al. 2008). Different formulations of the same drug may have different absorption characteristics. Manufacturing problems can and do occur. Pharmacy or pharmaceutical errors can alter the amount of drug delivered or, in fact, which drug is given, and parents or patients may or may not comply with instructions. All of these factors can alter the amount of drug the patient receives. Drug concentration measurements can identify or eliminate the uncertainty about a number of these factors in patients who have unusual or unexpected drug responses. However, patients who actually take or are given the same amount of a drug may also have very different amounts of drug in their body, blood, or site of action at different times after dosing. The ability to explain or predict the inter- and intraindividual differences in drug concentrations over time requires knowledge of some basic pharmacokinetics principles.

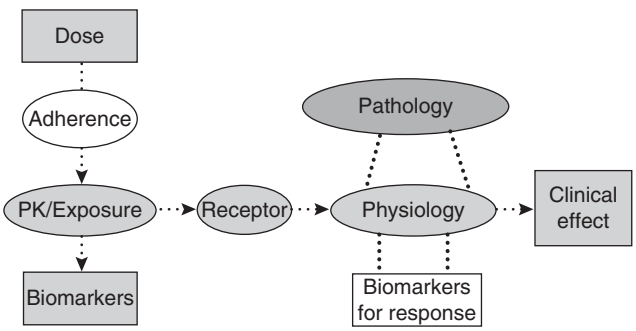

FIGURE 3.1 **Schematic representation of the interrelationship among drug input (dose), adherence, pharmacokinetics (concentration and exposure), receptor interaction, physiology/pathophysiology (pharmacodynamics), and clinical effects**. Examples of PK/exposure biomarkers are drug concentrations (total or unbound to plasma proteins) or activity (radiolabel or biological), whereas biomarkers for response can be a measurement of target enzyme activity/inhibition or response measurements, such as the international normalized ratio (INR) in anticoagulation therapy. Over time, the depicted processes (*oval symbols*) will be influenced by aging/development and disease progression. (Adapted from Kenna, L.A., Labbe, L., Barrett, J.S., & Pfister, M., 2005. Modeling and simulation of adherence: approaches and applications in therapeutics. *AAPS Journal* 7:E390–407, with permission of the publisher.

Much of our current knowledge of pediatric pharmacokinetics comes from the ability to measure serum concentrations of drugs, especially those with narrow therapeutic ranges or low safety margins. Therapeutic drug monitoring (or management) (TDM) of drugs has been done for years for drug classes such as antibiotics, anticonvulsants, and immunosuppressants. Measurements of psychoactive drug concentrations have given important insights into the different pharmacokinetics behavior in children and adolescents (Preskorn et al. 1986; Preskorn 1997).

Unfortunately, incorrect sample collection or analysis, or improper interpretation of results can diminish the value of TDM results. In addition, there are financial implications to the usefulness of TDM. Ample studies show that, when properly ordered, assayed, and interpreted, psychoactive drug TDM can occasionally be useful in all patients, and always in some clinical situations (Walson 1994).

For some psychotropic drugs (e.g., lithium and some antidepressants) a good association exists between plasma levels and therapeutic or toxic effects. Optimum steady-state levels can now be predicted from individual single-dose blood-level data of some drugs (lithium, nortriptyline, desipramine).

Altered pharmacokinetic behavior in children has to be taken into consideration in using psychotropic drugs. With development of suitable drug assays, plasma-level control of therapy is becoming an increasing part of good clinical practice. In fact, measurement of individual, genetically determined drug metabolizing potential, as well as individual drug receptor(s) and drug transporter(s) characteristics, is being used to prospectively predict drug dosing requirements as well as individual susceptibility to drug action, both therapeutic and toxic (de Leon et al. 2006; de Leon et al. 2008; Pestian et al. 2009; Prows et al. 2009).

The potential of psychoactive TDM to improve therapy has been limited mainly because many clinicians lack basic pharmacokinetics training, and pharmaceutical marketing departments actively discourage monitoring of their drugs. While the FDA has begun to require labeling statements about the usefulness of TDM, there is still much to be done to encourage TDM of psychoactive drugs as a way to improve individual therapy (Pichini et al. 2009). To improve this situation, clinicians must appreciate the basic pharmacokinetic principles necessary to correctly order and interpret TDM results, as well as to understand or predict drug actions in their individual patients.

This chapter gives a basic overview of pharmacokinetics and developmental aspects of pharmacokinetics. The review should allow clinicians to better understand drug disposition issues, so as to inform their ordering and interpretation of TDM, as well as help them to understand or design and interpret clinical drug trials.

PHARMACOKINETIC PRINCIPLES

The basic processes that control the concentration of drug at the site(s) of action resulting from a given dosage regimen include absorption, distribution, metabolism, and excretion. Collectively, these processes are referred to as ADME, and they provide an organized format for describing the pharmacokinetics of a particular drug and its dosage form. Absorption and distribution are primarily responsible for determining the speed of onset and magnitude of drug effect, whereas the processes of metabolism and excretion terminate the action of the pharmacologic agent by removing the active form of the drug from the body. Taken together, these four processes determine the duration of drug action (Rowland & Tozer 1995).

Drug absorption may occur from a variety of sites (e.g., gut, lung, nasal epithelium) by either active (e.g., transport-mediated) or passive mechanisms. The bioavailability (F) of a drug is the fraction that reaches the systemic circulation and is ultimately available to exert a biological effect on target tissues. This percentage is determined by the total amount absorbed and, for an orally administered drug, the metabolic elimination during first passage through the intestine and liver (first-pass effect). Absorption depends heavily on the route of entry, with intravenous administration producing the equivalent of 100% absorption (or F = 1). Oral administration is by far the most common route of administration, but also the most unpredictable in terms of final bioavailability. An orally administered drug is absorbed from the gastrointestinal tract and must first enter the portal circulation, where it passes through the liver before reaching the systemic circulation. During this first pass, a considerable portion of the drug is metabolized. Thus, only a fraction of the drug that was absorbed from the gastrointestinal tract enters the systemic circulation. The role of drug transporters, many of which show large, genetically determined individual and even developmental effects on the absorption of drugs from the gastrointestinal tract and active uptake (and efflux) into organs (e.g., liver, kidney, and brain) is being increasingly recognized (Cropp et al. 2008; Ho & Kim 2005; Murakami & Takano 2008; Wu & Benet 2005).

Most psychotropic medications are given orally and are absorbed after dissolution, primarily in the small intestine. For reasons of simplicity, the rate of absorption (expressed as a rate constant Ka [L/h]) is often assumed to be passive and obeying first-order principles, meaning that the rate of absorption is dependent on the amount of drug at the site of absorption, with a constant fraction absorbed per unit of time. It is important to realize that absorption is a rather complex process that is both rate- and time-dependent. So, Ka is not a constant, but rather a time-dependent absorption rate coefficient that changes as the drug is dissolved and absorbed from the lumen into the enterocytes.

An often-misunderstood principle is that concentration in the blood rises until the rate of absorption equals the rate at which drug is being removed from the body (the so called "peak"). This peak does not occur when absorption is complete but rather when the rate of absorption equals the rate of elimination. The time to peak is therefore determined by both absorption and elimination rates in the individual patient. Patients with faster elimination will have earlier peaks than will patients with slower elimination, even when the rate of drug absorption is the same (Fig. 3.2). The extent of absorption is usually expressed as the fraction absorbed or *bioavailability*. This is an important determinant of drug action. Although rate and extent of absorption are related, they are different. In general, the onset and magnitude of effects are related to the *rate* of absorption, whereas the average steady-state concentrations are related to the *extent* of absorption.

Once a drug is absorbed or enters the blood by direct injection, it is distributed throughout the body to various extravascular tissues. The pharmacokinetic term most often used to quantify the distribution of a drug throughout the body is the *apparent volume of distribution*. This volume does not necessarily correspond to a physiological space, and therefore, is preceded by the word "apparent." For most clinical applications, pharmacokinetic analysis can be simplified by representing drug distribution within the body by a single compartment in which drug concentrations are uniform (Rowland & Tozer 1995).

The relationship between the amount of drug absorbed (D), plasma concentration (Cp), and volume of distribution (Vd) can be summarized by the simple equation: Cp = D/Vd. Note that the larger the Vd, the

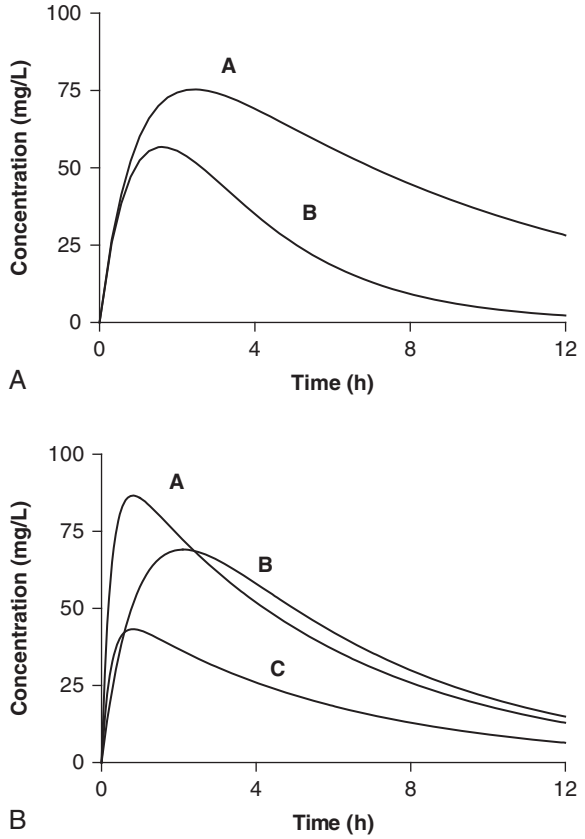

FIGURE 3.2 **Elimination versus Absorption rates. A:** Effect of differences in elimination rate on the maximum concentration (Cmax) and time to maximum concentration (Tmax) reached after a single oral dose. The drug absorption rate is the same (Ka = 1 h^{-1}) for curves A and B. The t1/2 for curve A is 6 h; the t1/2 for curve B is 2 h. **B:** Differences between absorption rates can have pronounced effect on the time (Tmax) at which the maximum concentration (Cmax) is reached. Curve A shows an orally administered drug with a four-times faster absorption rate as B. Curve C shows the effect of a 50% decrease in bioavailability of drug A. The half-life (4 h) is similar for scenarios A–C.

smaller the Cp. The Vd is the constant, which, when multiplied by the concentration gives the amount of drug in the body. The fact that it is not a true volume but a fictive volume, is best illustrated by the fact that some drugs have Vd's of many hundreds of liters thus exceeding the actual body weight by many times.

The two most important factors affecting distribution are fat stores and the relative proportion of total body water to extracellular water. The Vd of highly lipophilic drugs, including most neuroleptics and antidepressants, is affected substantially by the proportion of body fat.

At therapeutic concentrations, most psychotropic drugs follow first-order elimination or linear kinetics, in which a constant fraction of drug is eliminated independent of the amount circulating in the bloodstream. First-order kinetics implies a linear relationship exists between changes in dosage and the ln of the plasma concentration. Such a linear association allows for clinically relevant predictions of the impact of a dose change on circulating drug levels.

The pharmacokinetic term clearance (CL) best describes the efficiency of the elimination process. Clearance by an elimination organ (e.g., liver, kidney) is defined as the volume of blood, serum, or plasma that is totally "cleared" of drug per unit time. This term is additive; the total body or systemic clearance of a drug is equal to the sum of the clearances by individual eliminating organs. Usually, this is represented as the sum of renal and hepatic clearances: CL = CL renal + CL hepatic. Clearance is constant and independent of serum concentration for drugs that are eliminated by first-order processes, and therefore may be considered proportionally constant between the rate of drug elimination and serum concentration.

However, for some drugs (most notably phenytoin, salicylate, and ethanol) hepatic metabolizing enzymes become saturated even at normal therapeutic concentrations. The metabolism of other drugs can be saturated only in overdose situations. Saturation of drug metabolism occurs when relatively small concentrations of hepatic enzymes are available to metabolize these drugs (capacity-limited elimination). The elimination of these drugs is described by zero-order or nonlinear (Michaelis-Menten) kinetics. This process is characterized by the elimination of a constant amount of drug per unit of time, regardless of the plasma level. Certain psychotropic drugs, such as fluoxetine and nefazodone, have been observed to demonstrate zero-order kinetics at clinically relevant doses, making the relationship between dose changes and subsequent plasma levels much less predictable (Janicak et al. 1993). Nonlinear elimination and enzyme saturation resulting in decreased clearance may also occur at very high concentrations of any drug and should always be considered as a potential complication in case of intoxication.

For drugs that follow first-order kinetics, in addition to clearance, the *half-life* is a useful pharmacokinetic parameter to describe elimination. The elimination half-life ($t_{1/2}$) is the time required for the concentration of drug to decrease by 50%. In clinical practice, this parameter is referred to as the plasma (or serum) half-life and is usually assessed by measuring the fall of plasma or serum drug concentration and by graphically plotting the logarithm of the drug concentration versus time. The elimination half-life can be useful when determining frequency of dosing and dosing intervals. When a drug is dosed at regular intervals, it is the plasma half-life that determines the plasma *steady-state concentration* (Css). *Steady-state* is defined as an equilibrium between the amount of drug ingested and the amount of drug eliminated, resulting in no net change in plasma concentration over time. Steady-state (Css) is reached after approximately 4-5 times the half-life (Fig. 3.3).

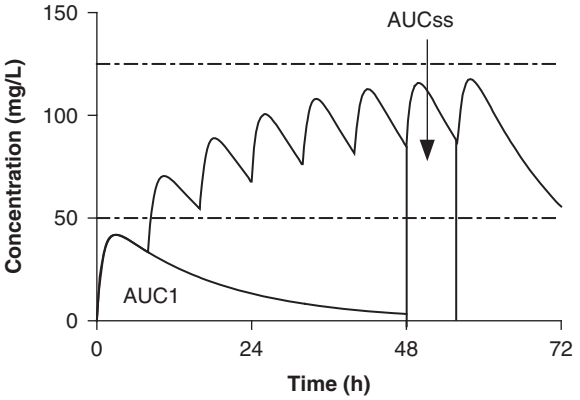

FIGURE 3.3 **Plasma concentration time profile of drug with a half-life of 4 hours administered every 8 hours.** Steady-state is reached after 4–5 times the half-life and *not* after three to four doses. The concentration time curve after the first dose shows the area under the curve (AUC1) after a single oral dose that is equivalent to the AUC at steady-state (AUCss). The broken lines represent the therapeutic range (arbitrary).

A common error is to confuse half-lives with number of doses. Unless given at an interval of a half-life, there is no relationship between number of doses given and steady-state. Conversely, if drug intake is stopped, it takes four to five half-lives (not doses missed) for drug elimination to be complete (Table 3.1).

Although elimination half-life is usually associated with clearance, it should be noted that this parameter is also influenced by distribution. This concept is important to appreciate when individualizing drug therapy, since it is clearance that determines steady state concentrations for any given dose absorbed.

Vignette 1: Blood Concentration of Phenytoin in hypoalbuminemia

An 8-year-old child with grand mal seizures experiences nystagmus while on 200 mg/day of long-term phenytoin treatment, given in three divided doses. A steady-state phenytoin concentration of 5.0 mg/L is measured (therapeutic range, 8–20 mg/L). Although other laboratory results were normal, it was noted that the child had profound hypoalbuminemia. Free (unbound) phenytoin concentration was 2.4 mg/L (therapeutic range 0.2–2 mg/L).

TABLE 3.1 *Fraction of drug eliminated and fraction remaining in the body as a function of the half-life*

Number of half-lives	Fraction eliminated (%)	Fraction remaining (%)
1	50.0	50.0
2	75.0	25.0
3	87.5	12.5
4	93.8	6.25
5	96.9	3.12

In this example, the increased unbound phenytoin concentration results in a larger volume of distribution (more free drug distributed to the tissues), increased clearance (more free drug available for metabolism), but no change in the drug's half-life. The paradox in this case is that the increased clearance caused the total phenytoin blood concentration to go down, while the free concentration was elevated and led to clinical toxicity. Moreover, the unchanged half-life would not have clarified the cause of the "subtherapeutic" phenytoin concentration.

PHARMACOKINETICS IN CHILDREN AND ADOLESCENTS

Children and adolescents display some unique pharmacokinetic properties; they are not "small adults" in the way that they handle drugs, and they do not necessarily require smaller doses (Kearns & Reed 1989; Kearns et al. 2003). Metabolic capacity at birth is lower than in adults, slowly increases to almost adult values between the age of 2 years and puberty, may exceed adult values during puberty, and then levels off to adult values with further aging and development (e.g., with neuroleptics, tricyclic antidepressants, pemoline, and methylphenidate) (Anderson & Holford 2008; Anderson & Holford 2009; Geller 1991). A comparable developmental process has been described for renal function maturation (Rhodin et al. 2009). Due to relatively more efficient renal elimination, children and adolescents will eliminate drugs that use renal pathways (e.g., lithium) more rapidly than will adults. Compared to adults, children have greater body water and relatively less adipose tissue. This can have a pronounced impact on drug distribution and accumulation of lipophilic drugs and metabolites. Therefore, the appropriate dose of a psychotropic medication in the pediatric age group should be empirically determined in combination with therapeutic drug monitoring. Dosing should not be solely based on adult extrapolations based on a proportion of body weight, as such an approach may lead to subtherapeutic concentrations. In addition, infants, children, and adolescents are not a homogenous group in terms of drug distribution patterns (Kearns & Reed 1989; Kearns et al. 2003; Morselli et al. 1980). These differences can be especially pronounced around the time of puberty, when hormonal changes can influence drug clearance and plasma drug concentrations achieved (Morselli & Pippinger 1982).

Although pharmacokinetic data in children and adolescents are limited, clinical observations suggest that children and adolescents require larger, weight-adjusted doses of most drugs than do adults to achieve comparable blood levels and therapeutic effects (Soldin & Steele 2000). This appears to be mostly the result of

increased rates of metabolism and elimination (Anderson & Holford 2008; Anderson & Holford 2009).

Developmental Factors Affecting Drug Disposition/Pharmacokinetics

Absorption and Bioavailability

Drugs are administrated by intravenous routes or extravascular routes including oral, sublingual, subcutaneous, intramuscular, rectal (by enema or suppository), inhalation, and transdermal. Available oral dosage forms include suspensions, immediate-release capsules or tablets, sustained-release capsules or tablets, and enteric-coated capsules or tablets that resist dissolution in the acidic pH of the stomach.

Drugs may be formulated as their salt forms (i.e., hydrochloride salt for base, sodium salts for acids) that dissociate in the body, or they may be formulated as the free acid or base. The fraction of the drug absorbed can be difficult to predict, as it is influenced by many factors. The extent and rate of absorption are partly determined by the physicochemical properties of the drug. Favorable absorption is related to lipid solubility, nonpolarity, and small molecular size. Reduced absorption is often observed for highly polar, nonlipid soluble, and large molecular size drugs.

Another important determinant of drug absorption is the degree of ionization, determined by the dissociation constant pK_a of the drug and the pH of the surrounding milieu. The gastrointestinal epithelium is more permeable to the nonionized form because this portion is usually lipid soluble and favors absorption. The degree of drug ionization will change as the pH increases from the stomach through the distal portion of the gut. The major factors influencing gastrointestinal absorption are pH-dependent diffusion and gastric emptying time. Slow gastric emptying time may retard drug absorption and can be rate-limiting. This is because the major absorption occurs in the proximal bowel, which has the greatest absorptive surface area. On the other hand, a slow transit time or slow intestinal motility may facilitate and prolong the absorption of some drugs. Gastric acid production and intestinal motility undergo substantial changes during the early stages of life but tend to be less different from adult values after the first year of life.

Little information is available regarding the effect of age or development on the absorption of psychotropic drugs (Kearns et al. 2003; Prandota 1985). This is further clouded by the paucity of drug formulations appropriate for children, which results in the crushing, mixing, or dissolving of adult formulations in various liquids and solids. This may alter both the rate and extent of drug absorption. Different foods or liquids used as a concomitant vehicle can have different pH values and thus may affect drug stability or absorption. Sustained-release products designed and tested in adults are a special problem. These dosage forms may not work the same in children whether they are crushed or not because of differences in gastrointestinal transit time. A product designed to release drug evenly over 24 hours simply will not work the same way in a child with decreased transit time, because only a fraction of the drug would be delivered. If a child was stabilized on a given dose and transit time, toxicity could be seen if the child became constipated, or a loss of efficacy seen if diarrhea developed. There are also developmental changes in diet, biliary secretion, and surface area, which could change the rate or extent of drug absorption. Maximum drug concentration "peaks" can occur sooner in children. This is often assumed to be the result of more rapid absorption; perhaps as a result of formulation differences. However, this may be the result of more rapid clearance rather than any difference in absorption rate. Since peak concentrations occur when the rate of absorption equals the rate of elimination (Fig. 3.2), in children with more rapid drug elimination, even when they have identical absorption rates, drug concentration will peak at an earlier time (but will also be lower as a consequence of higher clearance).

The role of active drug transport processes in both absorption and enterohepatic recycling of drugs has recently been shown to alter the pharmacokinetics of many drugs. *Enterohepatic recirculation* refers to the process by which drugs (or their conjugated metabolites) are secreted from the liver, where they are produced, into the bile and subsequently released into the small intestine, where they are reabsorbed into the general circulation (conjugated metabolites after cleavage conjugate by gut flora). Much remains to be learned about the developmental and pharmacogenetic control of such transport in individual responses to drugs.

Drug Distribution

After a drug is absorbed, it is distributed into various tissue compartments. The rate at which this occurs is determined by the blood flow to the tissues, as well as the rate of transfer of the drug from the blood into the tissues. This transfer depends on the vascular permeability to the drug, the relative binding of the drug to blood versus tissue components, the availability of active transport processes, involvement of efflux transporters, and the concentration gradient between blood and tissues. Many of these processes are altered during development. For as long as there is any drug in the body, drug distribution never stops; however, eventually the rates of transfer into and out of the tissues become equal. The time period prior to this equilibrium condition is called the *distribution phase*. Drug concentrations measured during the distribution phase will have a different relationship to drug action than will concentrations measured after distribution is complete. This is true for drugs with very slow or

rapid elimination. Appreciation of this principle is required to design studies to determine whether a concentration–effect relationship exists. For example, clonazepam studies that failed to find a concentration–effect relationship ignored the fact that clonazepam has an extensive, prolonged distribution phase (Greenblatt et al. 1987). The same patient can have distribution phase concentrations that are ten times predose concentrations. Concentrations drawn at random times after dosing would not be expected to relate to effects (Walson & Edge 1996). There is a common misconception that the distribution phase can be ignored for drugs, like clonazepam, which have slow elimination (i.e., long half-lives). Many centrally active drugs have prolonged distribution phases that must be appreciated to both properly interpret concentrations and design studies to investigate concentration/effect relationships (Preskorn et al. 1986).

Two important factors affecting distribution that change substantially during development are the amount of body fat and the relative proportion of total body water. For highly lipophilic drugs, such as most neuroleptics and antidepressants, the volume of distribution is greatly affected by the amount of body fat. The proportion of body fat is highest in the first year of life, followed by a steady decrease until an increase occurs at puberty (Milsap & Szefler 1986). Children and adolescents at different ages have varying degrees of fat stores, and in general tend to have a proportion of body fat less than that found in adults. Hence, in children one would expect to find a larger plasma concentration with lipophilic drugs when compared with adults after being given the same weight-adjusted dose. It has been demonstrated, however, that children actually exhibit a lower plasma concentration than do adults under these conditions. Therefore, other mechanisms (such as increased metabolism) must explain the lower plasma concentration of lipophilic drugs in children (Fetner & Geller 1992). The fact that certain psychoactive drugs can have profound effects on adiposity should produce temporal changes in individual responses to such drugs, especially in those patients who become obese.

The relative volume of extracellular water is high in children and tends to decrease with development. Extracellular water decreases gradually from about 40%–50% of body weight in the newborn to about 15%–20% by age 10–15 years. Total body water (TBW) decreases rapidly from about 85% of body weight in a small premature infant to about 70% in the full-term newborn to about 60% in the 1-year-old infant, and adult values (55% TBW) are gradually attained by 12 years of age. The percent of intracellular water remains stable from the first months of life through adulthood. Thus, drugs that are primarily distributed in body water (e.g., lithium) can be expected to have a lower plasma concentration in the pediatric population compared with that in adults because the volume of distribution is higher in children (Fetner & Geller 1992).

Protein Binding The amount of drug that binds to the receptor at the site of action determines the magnitude of drug effect. This in turn is determined by the amount and strength of drug binding to various proteins in blood and tissues. A major factor that affects distribution of a drug, and differs between pediatric patients and adults, is the extent to which the drug is bound to proteins (Grandison & Boudinot 2000). The amount of protein available, a drug's binding affinity for protein, and competition for binding with other endogenous and exogenous binding substrates all can impact the volume of distribution. Only the unbound drug is usually available to pass across membranes, distribute to extravascular tissues, and have pharmacological effects. Albumin is the primary plasma binding protein and, at the early stage of life, albumin concentration is directly related to gestational age. Albumin levels rapidly increase to adult levels by the end of the first year. Acid and neutral compounds bind primarily to albumin, which has high capacity to bind drugs. Many basic compounds bind also to the low-capacity binding alpha-1-acid glycoprotein (AAG). This protein is of particular importance, as its concentration in serum increases in conditions including stress, inflammation, and malignant disease. In general, changes in protein binding have the largest effects on drugs that are highly bound to blood proteins, or for drugs for which binding can be saturated at therapeutic concentrations (e.g., valproic acid; Herngren et al. 1991). Drug binding depends on the presence of other drugs or drug metabolites (displacement) (MacKichan 1989), and certain disease states (hepatic impairment, renal disease, malnutrition) (Blaschke 1977; Krishnaswamy 1989; Vanholder et al. 1993). For instance, the SSRIs fluoxetine, paroxetine and sertraline are highly protein bound to albumin and AAG. This raises the possibility of displacement interactions with other highly protein bound drugs. However, SSRIs are weakly bound to AAG. It appears that this is the reason why highly bound SSRIs do not increase the free fraction of other, concomitant highly bound drugs (Preskorn 1997). Drug protein binding may be reduced in young infants (Notarianni 1990) and certain disease states, but overall does not appear to be an important developmental factor in older children and adolescents (Grandison & Boudinot 2000).

Developmental or genetic changes in active drug transporter activities have not been well studied, but may also affect drug distribution and effects.

Drug Metabolism and Elimination Drugs have to exhibit a sufficient degree of lipid solubility (lipophilicity) in order to be orally absorbed and distributed to receptors

(e.g., in the central nervous system). For subsequent effective excretion from the body, conversion to more water-soluble (hydrophilicity) metabolites is required. The enzymes involved in this biotransformation are most highly concentrated in the liver, but also are present in several tissues throughout the body such as small intestine, lung, kidney, and adrenals. Most psychoactive drugs undergo extensive metabolism in the liver, although some may be excreted unchanged (e.g., lithium, gabapentin). The metabolic processes involved are categorized as either Phase I or Phase II reactions

Phase I metabolic reactions involve oxidation, reduction, or hydrolysis of the parent molecule resulting in the formation of a more polar compound (de Wildt et al. 1999a; Hines & McCarver 2002). Phase I reactions are mediated by the cytochrome P450 (CYP) family of enzymes. While metabolism used to be thought of as the body's detoxification process, phase I metabolites may be equally or even more pharmacologically active than the parent compound. Drug metabolism in general, and CYP 450-based mechanisms in particular, are discussed in detail in Chapter 4 (Oesterheld & Turpeinen).

Phase II metabolism involves conjugation with endogenous substrates such as sulfate, acetate or glucuronic acid (de Wildt et al. 1999b; McCarver & Hines 2002; de Wildt et al. 2009). Drugs may be conjugated directly or made more amenable to conjugation following the introduction of a functional group by phase I metabolism (e.g., hydroxylation). It is well recognized that the maturation of drug-metabolizing enzymes is a predominant factor responsible for age-related changes in metabolic drug clearance. For instance, neonates are deficient in many of the enzymes responsible for both phase I and phase II drug metabolism. Unfortunately, when unappreciated, these deficiencies have led to a number of adverse reactions such as the "gray baby" syndrome associated with chloramphenicol use (Weiss et al. 1960). Recent advances in our understanding of the ontogeny of the enzymes responsible for drug metabolism can explain past cases of drug toxicity in the neonate and help prevent such events in the future (de Wildt et al. 2009).

Developmental Aspects of Selected CYP Isoforms

With increasing knowledge of mammalian drug biotransformation processes, it has become apparent that not only are there developmental differences in expression among drug metabolizing enzyme families (cytochromes P450, glucuronosyl transferases etc.), but that individual drug metabolizing enzymes may have unique developmental profiles that influence the therapeutic response, to a given drug.

Knowledge regarding CYP ontogeny is quite limited, despite our increasing understanding of genetic variation in CYP activity in adults. For instance CYP2D6 phenotyping data suggest that enzyme activity in the newborn is markedly reduced, with activity gradually increasing during the first month of life to about 20% the adult activity level (Treluyer et al. 1991). In older children, CYP2D6 catalytic activity becomes comparable to that of adults around the age of 10 years but possibly much earlier, around the age of 3–5 years.

Based on available data, CYP3A4 activity is low at birth but increases rapidly during the early stages of life to reach approximately 50% of adult levels at ages 6–12 months, with a gradual increase to levels approximating those in the adult around the time of puberty (de Wildt et al. 1999a; Lacroix et al. 1997).

The implications of these changes depend on the specific CYP involved in a particular drug's clearance and on the number of alternative pathways, as well as induction or inhibition by other drugs. For instance, maturational changes in CYP3A4/5 activity are reflected by increased clearance in young children for midazolam and immunosuppressive drugs such as cyclosporine and tacrolimus (Zheng et al. 2003). As a consequence, higher dosages are required, as compared to adults, in order to reach the desired target blood concentrations.

THERAPEUTIC DRUG MONITORING

The developmental changes in pharmacokinetics of psychotropic medications that occur between birth and adolescence/adulthood create challenges for physicians who desire to prescribe medications on a rational, age-appropriate, individual basis. Routine therapeutic drug monitoring of prescribed drugs including active metabolite(s) can be of great help to individualize dose requirements during long-term treatment (Balant-Gorgia & Balant 1987; Balant-Gorgia & Balant 1992; Mitchell 2000; Pichini et al. 2009; Van Brunt 1983;). The ratio of metabolite to parent drug can also give important information on (non)compliance and can reveal unusual metabolic patterns.

Patients with lower or higher effects than expected, 3–4 weeks after initiation of therapy or after a dose change may benefit from TDM. In the nonresponding patient, concentration measurements will help the clinician in the decision to increase the dose or consider alternate therapy. Generally accepted indications for concentration measurements are summarized in Table 3.2.

Appropriate timing of drug concentration measurements is crucial for the appropriate interpretation of the level and subsequent dose adjustment. Within a dosing interval the predose or trough concentration is usually the most informative sampling time when steady-state is achieved but efficacy is questioned. In case of adverse events or (suspected) toxicity, sampling is preferably done at the time the side effects are experienced.

TABLE 3.2 *Indications for therapeutic drug monitoring of psychotropic drugs*

- Inadequate response
- Higher than standard dose required
- Serious or persistent side effects
- Suspected toxicity
- Suspected nonadherence
- Suspected drug–drug interactions
- New preparation, changing brands
- Other illnesses (e.g., hepatic/renal problems, inflammatory diseases)

Random sampling often results in not very useful drug monitoring, due to lack of the necessary information such as the actual time of drug intake, concomitant medications, and time of sampling. TDM laboratories can play an important role in improving the efficient use of TDM by providing up-to-date guidelines and teaching physicians about all the information necessary to interpret a result. This information must either be included in the laboratory requests or obtained by TDM or clinical pharmacokinetic monitoring services, such as those commonly run by clinical pharmacists and pharmacologists (Ensom et al. 1998; Murphy et al. 1996). Ideally, a pharmacokinetics interpretation should be reported back to the clinician along with the results.

For several drug classes, including psychotropic drugs, the use of population models and the application of a Bayesian optimization algorithm has been shown to be clinically useful and cost-effective (Burton et al. 1985; Tarright et al. 1994; Rousseau et al. 2000; van Lent-Evers et al. 1999). The method of Bayesian forecasting is derived from Bayes' theorem, and is based on the concept that prior pharmacokinetics knowledge of a drug, in the form of a population model, can be combined with individual patient data, such as drug concentrations (Jelliffe et al. 1998). The idea is to make an individualized model of the behavior of the drug in a particular patient, to see how the drug has been handled, and to obtain the necessary information to make rational dose adjustments so as to best achieve the selected target goal(s).

Vignette 2: Model-based, goal-oriented individualization of lithium therapy

A 17-year-old boy with bipolar disorder is started on lithium therapy, at 600 mg b.i.d. The initial lithium concentration is 0.8 mmol/L. As the patient's pressured speech and labile mood do not improve with time, the psychiatrist in charge wonders whether the lack of efficacy is due to insufficient coverage or to nonadherence. A repeat trough level is 0.3 mmol/L (Fig. 3.4a).

In this example, simulation of the lithium serum concentration profile based on population pharmacokinetics data reveals that this patient has a higher than normal clearance. Furthermore, the first level was not a trough level. The model-based profile and subsequent Bayesian individualization process are shown in Fig. 3.4b.

Bayesian methods can be more cost-effective than other techniques because they require fewer drug measurements for individual pharmacokinetics parameter estimation and can handle sparse and random samples (Merle & Mentre 1999). Therapeutic drug monitoring data, when applied appropriately, can also be used to detect and quantify clinically relevant drug–drug interactions (Gex-Fabry et al. 1997; Jerling et al. 1994).

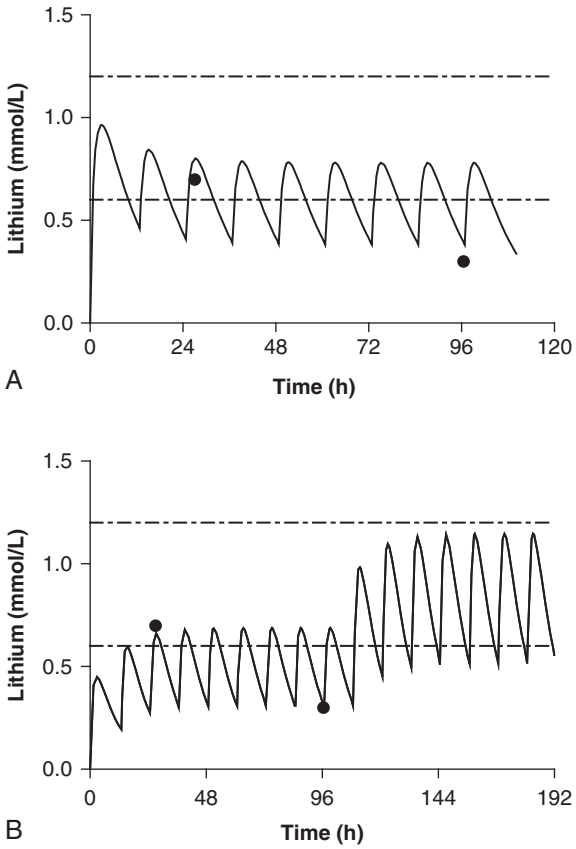

FIGURE 3.4 **Lithium plasma concentrations. A:** Lithium plasma concentration time profile based on a population PK model. The closed circles are the actual measured lithium concentrations. The first concentration was measured near the "peak" whereas the second was a trough. The broken lines represent the therapeutic range (0.6–1.2 mmol/L). **B:** Individualized lithium plasma concentration time profile based on the population model with feed-back of measured concentrations (Bayesian recalculation). The closed circles are the measured lithium concentrations. The second part of the curve is the predicted lithium concentration profile after increasing the dose to 1,000 mg lithium carbonate b.i.d., based on a target of 0.6–1.2 mmol/L (broken lines). The lithium population model used in Figure A is based on the model from Taright, N., Mentre, F., Mallet, A. & Jouvent, R., 1994. Nonparametric estimation of population characteristics of the kinetics of lithium from observational and experimental data: individualization of chronic dosing regimen using a new Bayesian approach. *Therapeutic Drug Monitoring* 16:258–69.

However, regardless of what pharmacokinetic dose individualization techniques are used, all are superior to a simple-minded comparison of a result to a "therapeutic range." Simply reporting results as being below, within, or above a published range is usually uninformative, not cost saving, and can lead to inappropriate actions.

CONCLUSION

Development can dramatically alter drug effects. In many cases, this is the result of pharmacokinetic changes. Appreciation of developmental pharmacokinetic principles will allow physicians to suspect and evaluate causes of unusual or unexpected drug effects. Often this will require drug concentration measurement and informed interpretation of the drug concentrations. As psychoactive drugs are studied more in children, the clinical and theoretical impact of developmental changes on drug kinetics and effects will be further elucidated. This should improve pediatric psychoactive drug therapy.

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4

Cytochrome P450-Mediated Drug Interactions

JESSICA R. OESTERHELD AND MIIA TURPEINEN

Twenty years ago, child psychiatrists' prescribing practices were often quite limited. They were generally free of concerns about possible drug–drug interactions. In the last 15 years, a number of factors have eroded this apparent complacency. Like their adult psychiatry colleagues (Frye et al., 2000; Rittmannsberger et al. 1999), child psychiatrists' prescribing patterns have shifted from monotherapy to co-pharmacy or polypharmacy (dosReis et al., 2005; Safer 1997; Staller et al. 2005). It has been estimated that 23%–42% of youth receiving psychiatric drugs are receiving multiple drugs (McIntyre & Jerrell 2009). For example, it is commonplace for a hospitalized teen to leave the hospital with an atypical antipsychotic, a mood stabilizer, an acne medication, or an oral contraceptive; or for a depressed latency-aged child who has asthma to be prescribed a selective serotonin reuptake inhibitor (SSRI), a hypnotic, and at least one antiasthmatic. Child psychiatrists have shifted from tricyclic antidepressants to SSRIs, drugs that are likely to produce drug interactions because they cause metabolic drug interactions via inhibition of hepatic cytochrome P450 (CYP) enzymes (Safer 1997). There has also been an increase in use by youth of nonprescription drugs (Adlaf et al. 2000), including drugs of abuse (Weir 2000), herbals (Walter & Rey 1999; Chapter x, this volume), and anabolic steroids (Evans 1997), and of prescription drug (Riggs 2008). Finally, there is a growing list of medications that have been withdrawn or severely restricted from the U.S. market because of their potential for fatal drug interactions; such drugs include terfenadine, astemizole, cerivastatin, mibefradil, and cisapride. Child psychiatrists have even been warned of possible serious drug interactions with drugs that they have used for years, such as pimozide (Flockhart et al., 2000; Friedman 1994; Horrigan & Barnhill 1994) and thioridazine (Carrillo et al., 1999; Maynard & Soni 1996).

Any attempt to memorize hundreds of potential drug interactions to prevent dangerous interactions is unproductive. Rather, a child psychiatrist should have a basic understanding of the types and timing of possible drug interactions and then develop prevention strategies in prescribing psychotropics. These may include the *personal formulary approach*, which is designed to enhance awareness of possible drug interactions of particular agents; the use of a *therapeutic alliance* with pharmacists, nurse practitioners, and other professionals to foster mutual cross-checking of drug-prescribing; and continuous review of updated software programs or Web sites each time one uses more than one drug (including nonpsychiatric ones).

The most commonly prescribed psychotropics currently in use in child psychiatry include (1) psychostimulants, (2) SSRIs, (3) mood stabilizers, (4) atypical antipsychotics, and (5) tricyclic antidepressants. Acceptance of atomoxetine in the treatment of attention-deficit hyperactivity disorder (ADHD) has reduced the use of central adrenergic agonists (Jensen et al. 1999; Johnston et al., 2006; Staller et al. 2005). Because many psychotropics are highly soluble and permeable, many of the drug interactions affecting SSRIs, tricyclic antidepressants, and antipsychotics occur during metabolism (i.e., via interactions with intestinal or hepatic CYPs or less often, with enzymes that carry out glucuronidation). Drugs with high permeability and low solubility (e.g., carbamazepine, digoxin) may be subject to these enzymes, but drug–drug interactions (DDIs) can also occur during absorption (i.e., in effects on drug transporters, such as P-glycoprotein [P-gp, ABCB1]). If clinicians understand the basics about these processes, they can use up-to-date CYP and P-gp tables that organize a vast amount of drug interaction data into simple groupings that allow a rational approach to prescribing. This chapter outlines such an approach, so

that child psychiatrists can develop a proactive stance toward possible interactions with drugs they prescribe. Vignettes will be presented to highlight clinically relevant aspects of CYP-based drug interactions. In addition, the authors will focus on central serotonin syndrome (CSS) as an example of the intertwining of CYP-based pharmacokinetic and pharmacodynamic factors that may occur during the use of psychotherapeutics in children.

PHARMACOKINETIC VERSUS PHARMACODYNAMIC INTERACTIONS

Drug interactions are classified as *pharmacokinetic* (i.e., occurring at sites prior to the engagement of drugs with receptors at their sites of action—during absorption, distribution, metabolism, or excretion) or *pharmacodynamic* (i.e., occurring at active sites), as illustrated in Figure 4.1.

An example of a pharmacodynamic drug interaction would be when a child develops a hyperserotonergic state with symptoms of nausea and myoclonus hours after tramadol (an analgesic opioid with pro-serotonergic properties) was added to a regimen of citalopram (another serotonergic agent). An example of a non–cytochrome-mediated pharmacokinetic drug interaction would be when a teenager who has had stable blood levels of lithium (a drug with a narrow therapeutic index) develops nausea, tremor, and slurred speech after taking ibuprofen for menstrual cramps, because of diminished renal lithium clearance (Ragheb 1990). This distinction is obviously artificial, since increases or decreases in drug levels are clinically meaningless unless they cause a pharmacodynamic effect (see Fig. 4.1).

PHASE I AND PHASE II METABOLISM

Most orally ingested drugs are absorbed from the small intestine before entering the portal vein on their way to the liver. Some drugs are substrates of efflux transporters, such as P-gp (see Table 4.1), which are present on the apical surface of the intestinal cell and act to block absorption of drugs from the gut. P-gp transport is saturable, and drugs that are both permeable and soluble easily saturate the transporter. These drugs rely primarily on metabolism. In the intestine, drugs that are permeable but poorly soluble may also rely on metabolism, but because of their poor solubility, they do not saturate the transporter and therefore can utilize P-gps. Digoxin is such a drug, and it is the standard P-gp substrate because it relies almost exclusively on transport rather than metabolism. P-gps are also involved in drug transport across the blood–brain barrier (BBB), and they facilitate drug elimination in the kidney and bile ducts (see Fig. 4.1). P-gp is also found at the maternal facing membrane of the placenta, and active efflux by

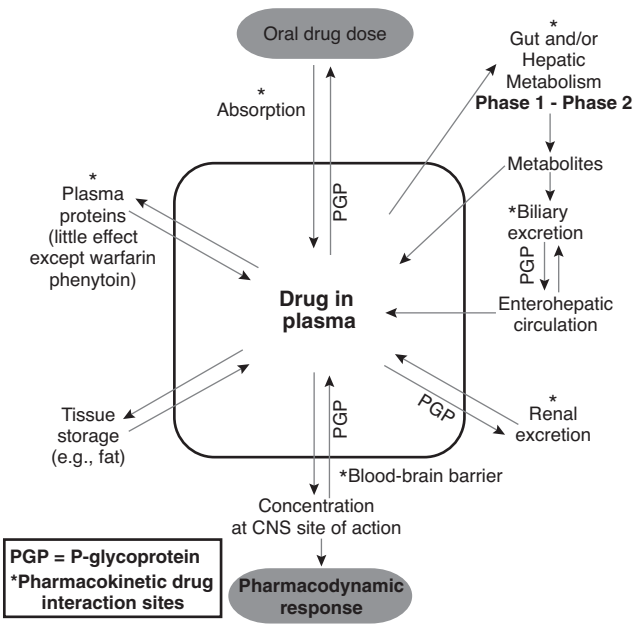

FIGURE 4.1 **Pharmacokinetics and pharmacodynamics of a central nervous system drug.** Adapted from Werry, J.S., & Aman, M.G., 1993. *Practitioner's guide to psychoactive drugs for children and adolescents*. New York and London: Plenum Medical Book Company, with permission of the publisher.

TABLE 4.1 *Some P-glycoprotein substrates, inhibitors, and inducers*

P-gp substrates	P-gp inhibitors	P-gp inducers
Cimetidine	Azithromycin	Dexamethasone**
Ciprofloxacin	Clarithromycin*	Phenytoin
Dexamethasone	Cyclosporine	Rifampin**
Digoxin	Erythromycin*	St. John's wort**
Erythromycin	Fluoxetine	
Fexofenadine	Grapefruit juice*	
Loperamide	Haloperidol	
Nortriptyline	Itraconazole*	
Risperidone	Ketoconazole*	
Sertraline	Olanzapine	
Talinolol	Quinidine*	
Topiramate	Propanolol	
Quinidine	Risperidone	
Verapamil		

For additional information, see http://www.genemedrx.com/PGPtable.php

*Drugs that are also CYP3A inhibitors

** Drugs that are also CYP3A inducers

P-gp restricts the maternal-to-fetal transfer of xenobiotics, protecting the developing fetus from drugs that the mother is exposed to (Behravan & Piquette-Miller 2007). The P-gp–based inhibition of absorption in the small intestine, kidney, and hepatic transport can itself be altered by other drugs, and a new category of drug interaction been described: interactions that occur through inhibition or induction of efflux transporters. Clearly, these pharmacokinetic changes are reflected in changed clearance of the victim drug (von Moltke & Greenblatt 2000; Westphal et al. 2000). Loperamide (an over-the-counter antidiarrheal opiate) has been shown in certain circumstances to cause respiratory depression when the central nervous system (CNS) is exposed to increased levels after BBB P-gp is blocked by quinidine (Sadeque et al. 2000). Not only may other opiates enter the CNS when coadministered with BBB P-gp inhibitors, but other psychiatric and nonpsychiatric drugs may cause CNS toxicity through this mechanism even with "normal" blood concentrations (Linnet and Ejsing 2008; von Moltke & Greenblatt 2000).

All plants, foods, and most drugs undergo processes that convert them from lipid-soluble to water-soluble agents to be inactivated. Exceptions include drugs that are already water-soluble: lithium (entirely) and gabapentin (mostly) are handled by the kidney. Most psychotropic drugs are metabolized in two steps by two distinct systems (see Fig. 4.2).

Phase I metabolic processes involve breaking off a part of the drug and inserting or uncovering a molecule (usually oxygen) to expose a chemical structure that serves as a "functional handle" for further phase II processing. The CYP enzymes are those generally responsible for phase I actions, and are primarily present in the gut and the liver (as well as in lung, kidney, brain, and other "barrier" tissues). Other phase I hydrolytic enzymes include plasma esterases, which are responsible for initiating metabolism of psychostimulants like cocaine (Warner & Norman 2000); microsomal epoxide hydrolases, which form the active metabolite of carbamazepine (Gilman 1991); monoamine oxidases, which besides deaminating several endogenous neurotransmitters like serotonin and norepinephrine, participate in the metabolism of levodopa and moclobemide; and flavin-containing monooxygenases, which are involved in the metabolism of clozapine and, to a lesser extent, olanzapine and chlorpromazine (Callaghan et al., 1999; Cashman et al. 1993; Tugnait et al. 1997).

Phase II reactions involve the formation of a link between the functionalized handle of a drug and a conjugate via a transferase. The most abundant conjugate is glucuronic acid, which is "hooked up" to the drug via a group of enzymes called uridine diphosphate glucuronyl transferases (UGTs). Unlike other phase II transferases that are in the cytoplasm, UGTs are microsomal and have close physical proximity to CYPs. A few drugs (e.g., lorazepam, oxazepam, temazepam, lamotrigine, and others) do not undergo phase I reactions because they already have "a chemical handle" (a hydroxyl group), and they are directly conjugated. Other phase II systems are *N*-acetyltransferases, which are known to acetylate clonazepam and phenelzine,

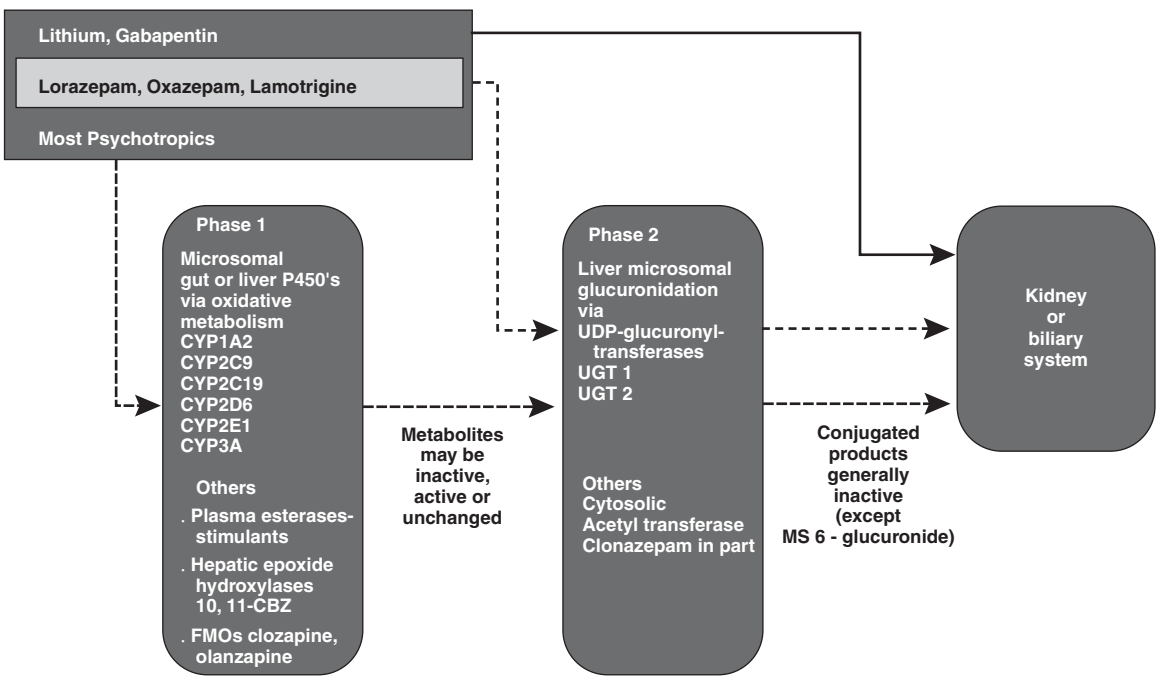

FIGURE 4.2 Phase 1 and phase 2 biotransformation.

and sulfotransferases, which conjugate acetaminophen. Phase I and phase II reactions are responsible for first-pass effect, and many psychotropic drugs have a substantial fraction removed prior to entry into the systemic circulation.

CYTOCHROME P450 ENZYMES

Cytochrome P450 enzymes are a superfamily of heme-containing enzymes. The metabolism of over 90% of all drugs is estimated to be mediated, at least partially, via CYPs (Pelkonen et al. 2008; Zanger et al. 2008). Their amino acid sequences have been determined, and a naming system has been developed on the basis of their amino acid similarities. The broadest group is stated first, with increasingly specific groupings designated in the nomenclature. Currently recognized families are named from 1 to 4, subfamilies from A to E, and specific enzymes are coded by specific genes from 1 on up. For example, CYP2D6 is an enzyme in the CYP2 family and D subfamily and is coded by gene 6. The CYPs that are near each other on the same gene and are related are grouped together (e.g., CYP3A4, CYP3A5, and CYP3A7) and are referred to collectively as CYP3A. There are 18 different known CYP-families and 42 subfamilies in humans covering 57 functional CYP genes (http://drnelson.utmem.edu/CytochromeP450.html). Enzymes in families 1–3 function mostly in the metabolism of a wide variety of xenobiotics like drugs, whereas other families are involved mainly in physiological functions such as the biosynthesis of fatty acids, steroid hormones, and bile acids (Nebert & Russell 2002; Pelkonen et al. 2008).

At this time, only three families of hepatic CYP enzymes have been shown to be involved in human metabolism of exogenous agents. The responsible hepatic CYPs include CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A. The ontogeny, genetics, and abundance of CYP enzymes that are most relevant to child psychiatrist are described in Table 4.2.

CYP3A is by far the single most important hepatic CYP family, since it metabolizes more than 40%–50% of drugs known to be metabolized by human CYP enzymes (de Wildt et al. 1999; Pelkonen et al. 2008). Few drugs are substrates of a single CYP (e.g., nefazodone by CYP3A, desipramine by CYP2D6). Most drugs are metabolized by several CYP enzymes. Although six CYP enzymes are found in the small intestine—CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP3A4, and CYP3A5—CYP3A4 is the lion's share of the total (Paine et al. 2006). Drugs or foods may be preferentially metabolized by small intestine or liver CYP3A. Drugs can also inhibit or block a CYP in either the small intestine (e.g., CYP3A by grapefruit juice or St. John's wort), the liver (e.g., CYP3A by nefazodone), or both.

A drug may be a substrate of one or two CYP enzymes and inhibitor of none (e.g., quetiapine is a substrate of CYP3A, and it is not a CYP inhibitor); or a substrate of one and an inhibitor of the same CYP (e.g., nefazodone of CYP3A); or a substrate of one CYP and an inhibitor of another CYP (e.g., quinidine is a substrate of CYP3A and an inhibitor of CYP2D6). All individuals in some classes of drugs are metabolized by the same CYP, such as nonsteroidal anti-inflammatory drugs (e.g., [NSAIDs] and COX-2 inhibitors by CYP2C9), and some members of some classes of drugs are metabolized by different CYPs (e.g., see Table 4.3. for pathways and inhibition of various SSRIs).

A classification system similar to that for the CYPs has been developed to characterize the UGT superfamily. There are two clinically significant hepatic UGT subfamilies (UGT1A and UGT2B), and UGT1A1 is the workhorse of this system (Tukey & Strassburg 2000). See http://www.genemedrx.com/UGTtable.php for a more complete list.

Mechanism of Cytochrome P450 Involvement in Drug Interactions

If one drug blocks one or more CYP enzymes and another drug is added that is metabolized only by the blocked CYP, then more of the second drug enters the systemic circulation unmetabolized, and its circulating levels are increased. This effect is amplified with continued dosing of the combination.

> Vignette 4.1 Blood Concentration of Substrates Is Increased by Cytochrome P450 Inhibition
>
> A child with Tourette disorder is treated with a daily dose of 2 mg of pimozide for tics after failure of several atypical antipsychotics. A family doctor treats a streptococcal pharyngitis with clarithromycin, and 24 hours later the child develops palpitations. An electrocardiogram (EKG) reveals a QTc prolongation to 0.465 milliseconds.

In this example, clarithromycin, a potent CYP3A inhibitor, blocks the principal pathway of pimozide metabolism (CYP3A), and plasma concentrations of pimozide increase. A higher pimozide concentration (a pharmacokinetic effect) is associated with prolongation of QTc in EKG readings and potentially fatal torsades de pointes (via potassium channel blockade, a pharmacodynamic effect; see Flockhart et al. 2000, Mayhew et al. 2000). As exemplified by this vignette, the pharmacokinetic effect of inhibition occurs relatively quickly, since ongoing metabolic processes are interrupted.

> Vignette 4.2 Blood Concentrations of Active Metabolites of Substrate Pro-drugs Are Reduced by Cytochrome P450 Inhibitors

TABLE 4.2 *Hepatic Cytochrome P450 Characteristics*

	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4	CYP3A5	CYP3A7
Chromosome	15	19	10	10	22	10	7	7	7
Ontogeny	Onset: 3 months	Onset: After birth	Present in fetus	Present in fetus	Present in fetus	Onset: After birth	Onset: After birth	Present in fetus	Major fetal CYP
Development*	20-25% at 3-12 months 50% at 1 year and in children, activity exceeds adult levels	50% by 1 year	20% at birth, 50 % by 1 month and in children, activity exceeds adult levels	30% at birth, linear increase first 5 months 100% by 1 year	30% at 7-28 days 70% at 4 weeks-5 years	30% at 3-12 months 100% at 1-10 years	Increases steadily over first year 70% at 1 year and in children, activity exceeds adult levels	Remains in a small percentage of adults with CYP3A5*1	Decreases over first year and remains in small percentage of adults with CYP3A7*1c
Content of total CYPs	~10%	<5%	~15%	<5%	<5%	~15%	~30%		
Genetics		Polymorphisms Poor metabolizer: African 33%-50% Asian 17% White 25%	Polymorphisms Poor metabolizer: <2% all races	Polymorphisms Poor metabolizer: African 4%-7% Asian 12%-22% White 3%-5%	Polymorphisms Poor metabolizer: African 7%-10% Asian 1% White 7%-10% Ultrarapid metabolizer: Ethiopian 30% White 1%-3.5%				

Legend: P450 cytochrome= CYP, *= amounts given in percentage of adult values
from Anderson & Lynn 2009, Croom et al. 2009, Hines 2007, Zanger et al. 2008

A 17-year-old with major depressive disorder is treated with fluoxetine at 20 mg/day. She has a ligament injury while playing soccer. Fluoxetine is discontinued 5 days before surgery. After surgery, she is given oxycodone and continues to complain of pain.

This vignette illustrates a different aspect of CYP inhibition. Oxycodone, hydrocodone, and codeine are all pro-drugs that are converted to active analgesics by CYP2D6 metabolism. If their conversion is blocked by co-prescribed drugs, less analgesia will result. In this example, although fluoxetine itself had been discontinued, its long-lived metabolite, norfluoxetine, was still present, and it inhibited the conversion of the pro-drug oxycodone to its active metabolite.

Types of Cytochrome P450 Inhibitors

There are three biochemical mechanisms of CYP inhibition: competitive, mechanism-based, and metabolite–intermediate complex (Fig. 4.3). Each type of inhibitor

TABLE 4.3 *Cytochrome P450 drug interaction table*

Substrates

CYP1A2	CYP2B6	CYP2C19	CYP2C9	CYP2D6	CYP3A4,5,7	
Psychotropics: amitriptyline chlorpromazine clomipramine clozapine duloxetine fluvoxamine imipramine melatonin mirtazapine olanzapine ramelteon thioridazine **Others:** caffeine cyclobenzaprine frovatriptan naproxen ondansetron theophylline tizanidine *verapamil* zileuton zolmitriptan	artemisinin bupropion ketamine meperidine mephobarbital sertraline	**Psychotropics:** amitriptyline citalopram clomipramine diazepam escitalopram flunitrazapam {fluoxetine} imipramine moclobemide sertraline trimipramine **Anticonvulsants:** mephenytoin phenytoin **Proton Pump Inhibitors:** {esomeprazole} lansoprazole omeprazole {pantoprazole} **Others:** carisoprodol cyclophosphophamide (p) nelfinavir proguanil (p) propranolol R-warfarin voriconazole	**Psychotropics:** fluoxetine {sertraline} valproic acid **NSAIDS:** celecoxib diclofenac flurbiprofen ibuprofen indomethacin lornoxicam meloxicam {naproxen} piroxicam suprofen tenoxicam **Hypoglycemics:** chlorpropamide glipizide glimepiride glyburide nateglinide *{rosiglitazone* tolbutamide **Others:** bosentan candesartan fluvastatin irbesartan losartan (p) phenobarbital phenytoin sulfa drugs S-warfarin tetrahydro-cannabinol (marijuana) torsemide *zafirlukast*	**Psychotropics:** amphetamines amitriptyline aripiprazole atomoxetine benztropine chlorpromazine clomipramine desipramine doxepin duloxetine fluoxetine fluvoxamine haloperidol imipramine mirtazapine nortripyline paroxetine perazine perphenazine risperidone {sertraline} thioridazine venlafaxine **Antihistamines:** chlorpheniramine diphenhydramine **Beta Blockers:** alprenolol carvedilol metoprolol propranolol timolol **Opiates:** codeine(p) hydrocodone (p) {oxycodone} tramadol **Others:** dextromethorphan in cough syrup dolasetron(p) encainide flecainide MDMA (Ecstasy) metoclopramide propafenone tolterodine	**Psychotropics:** alprazolam amitriptyline aripiprazole buspirone carbamazepine citalopram {clomipramine} {clozapine} {diazepam} escitalopram estazolam eszopiclone fluoxetine haloperidol midazolam nefazodone pimozide quetiapine risperidone sertraline trazodone triazolam zaleplon {ziprasidone} zolpidem **Drugs of abuse/ treatment:** alfentanil buprenorphine cocaine fentanyl ketamine methadone oxycodone phencyclidine **Antibiotics/ Antifungals:** macrolides (not azithromycin) itraconazole ketoconazole telithromycin **Anticonvulsants:** carbamazepine ethosuximide felbamate tiagabine zonisamide	**Asthma Medication:** fluticasone salmeterol zileuton **Calcium Channel Blockers** amlodipine diltiazem felodipine nifedipine verapamil **Hormones/ chemotherapeutics:** cortisols cyclosporine desogestrel (p) doxorubicin ethinyl estradiol *OCs* progestins/progesterone tacrolimus tensirolimus vincristine and others **HIV:** indinavir ritonavir saquinavir **Others:** aprepitant artemisinin cilostazol cinacalcet granisetron imatinib *Irinotecan* *lidocaine* nateglinide *ondansetron* PPDE5 Inhibitors (sildenafil, tadalfil, vardenafil) quinidine Statins (lovastatin, atorvastatin, simvastatin) sunitinib

{ } minor pathway, (p) pro-drug

(*Continued*)

TABLE 4.3 *Cytochrome P450 drug interaction table (Continued)*

Inhibitors

CYP1A2	CYP2B6	2C8	CYP2C19	CYP2C9	CYP2D6	CYP3A4,5,7
caffeine	efavirenz	**gemfibrozil**	chloramphenicol	amiodarone	amiodarone	amiodarone
cimetidine	fluoxetine	glitazones	delavirdine	anastrazole	amitriptyline	amprenavir
ciprofloxacin	fluvoxamine	montelukast	efavirenz	delavirdine	**bupropion**	aprepitant-initially
enoxacin	ketoconazole	trimethoprim	felbamate	efavirenz	celecoxib	**atazanavir**
echinacea	nelfinavir		fluconazole	fluconazole	chlorpheniramine	cimetidine
fluvoxamine	oral contraceptives		fluoxetine	fluoxetine	chlorpromazine	ciprofloxacin
norfloxacin	paroxetine		fluvoxamine	fluvoxamine	cimetidine	**clarithromycin**
ofloxacin	ritonavir		isoniazid	fluvastatin	**cinacalcet**	**delavirdine**
oral contraceptives	thiotepa		lansoprazole	isoniazid	citalopram	diltiazem
rofecoxib			modafinil	phenylbutazone	chlorpheniramine	doxycycline
verapamil			omeprazole	sertraline	clomipramine	echinacea
zileuton			oral contraceptives	sulfamethoxazole	cocaine	enoxacin
			oxcarbazepine	**sulfaphenazole**	desipramine	erythromycin
			topiramate	teniposide	diphenhydramine	fluconazole
			voriconazole	valproic acid	doxepin	fluvoxamine
				voriconazole	duloxetine	grapefruit juice
				zafirlukast	escitalopram	imatinib
					fluvoxamine	**indinavir**
					fluoxetine	**itraconazole**
					goldenseal	**ketoconazole**
					halofantrine	miconazole
					haloperidol	**nefazodone**
					hydroxyzine	**nelfinavir**
					imipramine	**norfloxacin**
					metoclopramide	**ritonavir and boosted PIs**
					moclobemide	**saquinavir**
					paroxetine	star fruit
					perphenazine	**telithromycin**
					pimozide	verapamil
					propafenone	**voriconazole**
					quinidine/quinine	
					ritonavir	
					sertraline	
					terbinafine	
					thioridazine	

Bold: potent inhibitor

Inducers

CYP1A2	CYP2B6	2C8	CYP2C19	CYP2C9	CYP2D6	CYP3A4,5,7
carbamazepine	lopinavir/ritonavir	rifampin	gingko biloba	aprepitant-long term		aprepitant long-term
char-broiled meat	phenobarbital		rifampin	barbiturates		barbiturates
cigarette smoke	phenytoin		St John's wort	bosentan		bosentan
cruciferous veggies	rifampin			carbamazepine		carbamazepine
esomeprazole	ritonavir			lopinavir/ritonavir		efavirenz
lansoprazole				rifampin		felbamate
marijuana smoke				ritonavir		glucocorticoids
omeprazole				St John's wort-long term		modafinil at >200 mg/day
rifampin						nafcillin
ritonavir						nevirapine
						(oxcarbazepine)
						phenytoin
						primidone
						rifampin
						St John's wort
						pioglitazone
						topiramate at >200 mg/day

( ) modest inducer

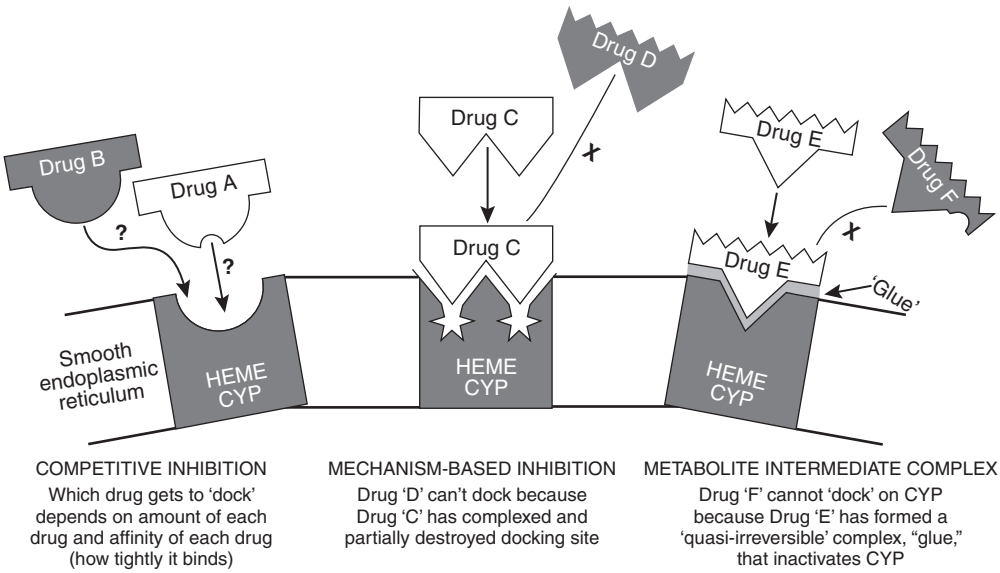

FIGURE 4.3 Different types of CYP inhibitors.

differs in the nature of CYP binding. Competitive inhibitors are reversibly bound and can be "competed off" of the docking site if another substrate of higher affinity is present at a higher concentration. Therefore, drug interactions are more likely to occur with drugs of the highest affinity (and lowest *K*is, e.g., ketoconazole and related compounds are among the most potent competitive inhibitors). Metabolite–intermediate complex inhibitors bind very tightly to the CYP but do not destroy the docking site, as do mechanism-based inhibitors. Since neither of these latter types of inhibitors can be pushed off the site by other drugs, these types of inhibitors are more likely to cause clinically significant interactions than are drugs that are low-affinity competitive inhibitors (e.g., in Vignette 4.1, clarithromycin is a metabolite–intermediate-complex inhibitor).

Intestinal P-glycoprotein and CYP3A as Partners in Cytochrome P450 Inhibition There is enormous interindividual variation in the amount of both intestinal CYPs and P-gp present (Hebert 1997; Paine et al. 1997). P-glycoprotein acts as a gatekeeper to a drug's contact with intestinal CYP enzymes. P-gp and CYP3A4 can function in a complementary fashion to reduce a drug's entry through the intestine, and the regulation of their activity is coordinated through the Pregnane-X receptor and others (Hariparsad et al. 2009). It is clear that some drugs are both P-gp inhibitors and CYP3A inhibitors (e.g., erythromycin, ketoconazole, quinidine; see Table 4.1). The blood concentration of vulnerable substrates can be dramatically increased by "double inhibitors," and these drug pairs seem more likely to produce clinically significant drug interactions (see Table 4.1).

Cytochrome P450 Inhibition and Clearance of Drugs Metabolized by the Liver Clinicians should note that if a drug has high presystemic clearance (i.e., most of the drug is metabolized before reaching the systemic circulation) and an inhibitor of its metabolism is added, the interaction that results may well be characterized by an immediately elevated plasma drug concentration of the substrate drug (e.g., triazolam is metabolized extensively by CYP3A and ketoconazole has an immediate effect on its concentration; see Greenblatt et al. 1998). An increase in blood concentrations may result in the *immediate* development of symptoms and signs of benzodiazepine toxicity (e.g., increased sedation, impaired psychomotor coordination, and confusion). However, if a drug has a low presystemic clearance (i.e., little of the drug is metabolized by CYP3A before reaching the systemic circulation) and an inhibitor of its metabolism is added, little change in plasma drug concentration will occur after the first pass. It is only with repeated drug administration of both drugs that a cumulative effect will be evident (e.g., alprazolam and ketoconazole will result in little initial benzodiazepine toxicity, but toxicity may develop over a week). This distinction is important for clinicians, as two patterns of drug interaction are evident: those occurring *immediately* after the addition of a second drug, and those occurring *later*, when the second drug has reached steady state.

Cytochrome P450 Induction

Some drugs can also increase the amount of active CYP enzyme via effects on protein stabilization or the transcriptional apparatus. This process is called *induction*.