PART I

FUNDAMENTALS AND CONCEPTS

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CURRENT NEEDS FOR NEW THERAPEUTIC AGENTS AND DISCOVERY STRATEGIES—A SYSTEMS PHARMACOLOGY APPROACH

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1.1 INTRODUCTION: A BRIEF HISTORY OF DRUG DISCOVERY

The use of remedies to treat or alleviate symptoms of a medical condition can be traced as far back as ancient Egypt. The Ebers papyrus, dating from 1555 BC, was found to contain 876 concoctions to treat a wide variety of disorders [1]. Early medicinal efforts were also used by the Greeks, most notably Hippocrates, and by several Asian cultures including the Chinese [2]. However, the identification of active ingredients and the development of the interdisciplinary science of pharmacology that bridged organic chemistry, zoology, and pharmacology did not emerge until the late 1800's. These advances were made possible by progress in chemistry, including theories on acids and bases and on the structure of aromatic molecules such as dyes [3]. In the 1870's, Paul Ehrlich proposed the existence of "chemoreceptors", which differed between microorganisms and the host tissue, based on his studies of dyes in biological tissues [3]. He suggested these could be used therapeutically, which eventually gave rise to the development of a class of drug treatments known as chemotherapy.

At the beginning of the twentieth century, pharmacology progressed quickly. In 1905, J. N. Langley introduced the concept of a "receptive substance", the modern basis for the study of receptor agonists and antagonists [4, 5]. In 1933, Meldrum and Roughton identified the enzyme carbonic anhydrase while studying the effects of sulfanilamide, the active metabolite of the antibiotic sulfamidochrysoidine [6]. This discovery led to the concept of enzymes as a good target for drug discovery and gave further importance to the biochemical characterization of cellular functions. Following Alexander Fleming's discovery of penicillin as a product from the *Penicilium* mold that killed *Staphylococcus* bacteria [7], many drug companies invested in microbiology, resulting in the discovery of more antibiotic and other therapeutic agents [3].

Over the next decades, drug discovery progressed along with our understanding of the basic sciences that underlie the discipline of pharmacology. What is currently referred to as interdisciplinary and translational basic science became known as pharmacology and experimental therapeutics. The large explosion of chemical libraries in the 1980's by the large scale implementation of combinatorial chemistry required the simultaneous development of high-throughput screening (HTS) methodologies. With these new technologies, many hundreds of thousands of compounds could be synthesized and then screened against a target of interest. However, this approach was often largely detached from a physiologically relevant screening signal. Another major breakthrough that changed the way society as

Development of Therapeutic Agents Handbook, First Edition. Edited by Shayne Cox Gad.

well as pharmacologists thought about therapeutics coincided with the development of the human genome project in 1986. The hope that a large number of drug targets would soon be identified and, in combination with the HTS technology, that a rapid increase in the discovery of therapeutic agents would result [8] effused through the pharmacology and biomedical sciences community as well as Wall Street aficionados. However, this original optimism met with a declining number of drugs approved by the U.S. Food and Drug Administration (FDA) since 1996, seeming to suggest that a target-based random screening approach was not the panacea originally envisaged for drug discovery.

In this chapter, we will provide a brief overview of some current therapeutic agents, including their weaknesses, and highlight selected opportunities for continued drug discovery efforts in certain disease areas. We will then focus our attention on different strategies used to identify new therapeutic agents, comparing target-based discovery (TBD) and what we refer to as systems-based discovery (SBD). Although pharmaceutical companies have favored TBD during the past thirty years, emerging evidence indicates that SBD may help in solving some of the issues that have plagued the modern approach to drug discovery. Finally, we will explore the challenges associated with the application of SBD to neurological disorders. This chapter is intended neither to be exhaustive or fair and balanced but presents a selective viewpoint. We apologize to those pioneers whose work we may have failed to cite and for simplifications we introduced in the interest of streamlining this chapter.

1.2 CURRENT STATE OF THERAPEUTIC AGENTS AND THE NEED FOR NEW AGENTS

1.2.1 Overview of the Current State of Therapeutic Agents

Therapeutic agents currently on the market can be placed into either one of four categories: small molecules, which are chemically synthesized compounds such as aspirin; natural products, which are small, naturally occurring molecules that are generally isolated from plants, fungi and mold (such as penicillin); biotherapeutics, which are macromolecules that occur naturally (such as insulin and tissue plasminogen activator) or are engineered based upon a biological template (such as trastuzumab (Herceptin, Genentech), a monoclonal antibody designed to treat HER2-positive breats cancer); and nucleic-acid-based therapeutics, which are deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules designed to interfere with the stability or translation of a messenger RNA (mRNA) (such as fomivirsen (Vitravene, ISIS Pharmaceutical and Novartis), an antisense molecule designed to treat cytomegalovirus infections in the retina of immunocompromised patients). The decision to use a small molecule or a biotherapeutic agent is generally dictated by the desired target and, as discussed below, these two classes of therapeutic agents complement each other in terms of their respective capabilities and weaknesses.

Small Molecules and Natural Products For drug discovery, small molecules are typically less than 500 molecular weight (MW) units, although this is not an absolute cut-off as molecules of higher molecular weight could be acceptable if sufficient bioavailability could be achieved. Ultimately, most small molecules are chemically synthesized, but their discovery may be the result of different strategies. Historically, most small molecules were initially isolated from natural products, which is now in disfavored due to the unfavorable logistics of developing commercially viable large-scale chemical syntheses of complex natural products. For example, the compound paclitaxel (Taxol, Bristol-Myers-Squib) was isolated from the bark of a North American yew in the 1960's (Fig 1.1) [9]. It was later shown to have novel anticancer properties by binding to tubulin, resulting in the stabilization of microtubules and disruption of mitosis [10]. However, the treatment of one cancer patient would have required the harvesting of six yew trees. To address this issue, a synthesis scheme was eventually developed using an analog of paclitaxel,

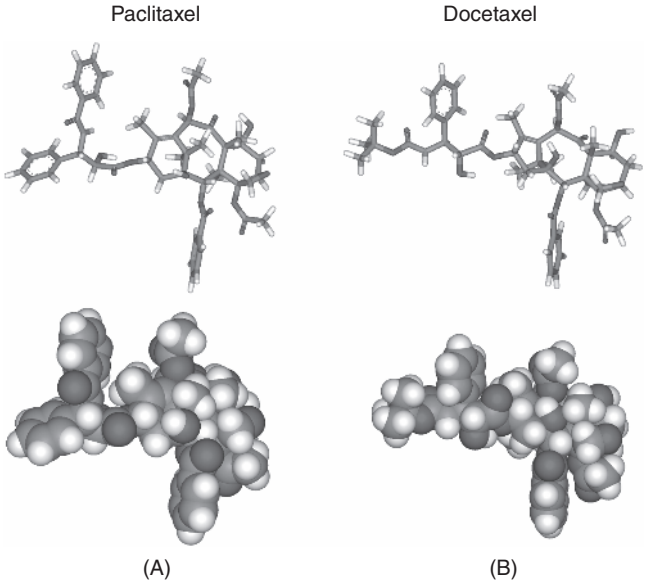

Figure 1.1 Chemical structure of (A) paclitaxel and (B) docetaxel.

10-deacetylbaccatin III (DAB), as starting material (reviewed in ref. [11]). DAB was obtained from the needles of European and Himalayan yew trees, avoiding the harvesting of trees. As shown by this example, isolation of a natural substance may require large amounts of starting material to identify and isolate sufficient quantities of the active ingredient. However, the effort and development costs were clearly justified in this case by the discovery of this life-saving anti-cancer agent. A second and important added value is that the advances in science resulting from the study of natural products may also lead to a drug that acts via a new mechanism and thereby initiate a new area of research on the disease biology.

Iterative modification is a strategy in which novel small molecules are synthesized based upon a well-known template until a novel structure, with improved properties and/or efficacy is obtained. Such an approach can lead to the production of a substantiative patent estate. This is the case with docetaxel (Taxotere), an analog of paclitaxel developed by Potier and colleagues at the Centre national de la recherche scientifique (CNRS) [12] and commercialized by Sanofi Aventis (Fig 1.1). Docetaxel was identified in a structure–activity relationship campaign where paclitaxel derivatives were tested in a microtubule depolarization assay [12]. In this in vitr*o* assay, docetaxel was about twice as potent as paclitaxel. When compared to paclitaxel in different cell line models of tumor, docetaxel was 1.3- to 12-fold more potent and this was found to be due to docetaxel's higher affinity for microtubules [13]. Further studies using in vivo xenograft models revealed that docetaxel was as effective as or more effective than paclitaxel [14]. In addition, docetaxel treated tumors, such as the C38 colon adenocarcinoma, in which paclitaxel was ineffective. Aside from its higher potency and broader efficacy spectrum, docetaxel is also an improvement over paclitaxel in that it is more soluble [12] and is considered schedule independent [14]. This example demonstrates how structure–activity relationship studies based on variants of a known drug can give rise to novel, improved small molecules.

With advances in the technology for characterization of the three-dimensional structure of proteins, the concept that the design of small molecules could be guided by the structure of the target recognition site(s) emerged. Information required to deduce the structure of the active site and other binding pockets can be obtained using X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. Candidate molecules may then be designed to interact with specific amino acids at the desired site of action using molecular modeling, bioinformatics, and computational chemistry. In the final steps of the lead identification process, compounds can be modified iteratively to improve upon or optimized the initial lead's chemical properties, efficacy, and/or potency. This process of "rational drug design" resulted in much enthusiasm during the 1980's and early 1990's but seemed to become incorporated with the platform technology of medicinal chemisty rather than the driving force behind new pharmacological discovery.

The Bcr-Abl kinase inhibitor imatinib mesylate (Gleevec, Novartis) is considered to be the first drug designed rationally [15, 16]. Chronic myeloid leukemia is the result of a reciprocal translocation of chromosomes 9 and 22 which creates a gene encoding for the Bcr-Abl kinase, a highly active tyrosine kinase [17, 18]. The design of a specific Bcr-Abl kinase inhibitor takes advantage of the fact that, although several kinases display sequence homology and similar active conformations, the kinases differ in the conformation of their inactive state [19]. Hence, a specific inhibitor could be designed to stabilize the enzyme's inactive conformation, which is different from that of other kinases, and act by preventing phosphorylation of the substrate(s). The crystal structure of Bcr-Abl kinase bound to an analog of imatinib mesylate appeared to reveal an atomic basis for the specificity of interaction [19]. The compound and Bcr-Abl kinase form a number of hydrogen bonds and van der Waals interactions, which are not possible with other kinases. Interestingly, imatinib mesylate interacts specifically with the inactive conformation of the Bcr-Abl kinase as activation causes a conformational change in the kinase that prevents the drug from binding.

During the development of small molecules therapeutics, optimization is geared toward obtaining a desirable set of physico-chemical properties. At the molecular level, these generally include potency and efficacy and, in most cases, specificity for a particular target. At the level of the whole organism, low toxicity (at an efficacious dose) and adequate pharmacodynamic and pharmacokinetic properties must be achieved. For most therapeutic candidates, the ability of the compound to cross the blood–brain barrier (BBB) and blood–nerve fiber barrier (BNFB) must also be taken into consideration. Nervous tissues are very effectively shielded from endogenous and exogenous molecules, whether centrally or peripherally, through the use of physical, biochemical, and cell biological mechanisms (reviewed in ref. [20]). The endothelial cells lining the intracerebral capillary walls are unusual in that they are connected by tight junctions forming patent zonula occludens and lack fenestrations. In addition, these endothelial cells possess metabolic enzymes and transmembrane transporters that further decrease the penetration of most drugs. Small molecules may cross the BBB with both passive or active mechanisms, as well as vesicular

trancellular mechanisms. For passive transport, the size of the molecule, its lipophilicity and charge (pK_a) are the main determinants. Drug candidates with specific functional groups may also be actively taken into the brain via transporters. In some cases, however, BBB integrity may be disrupted, facilitating the penetration of small and large molecules. This is seen most often following pathological conditions, such as stroke, trauma, infections, and neuroinflammatory diseases.

Over the course of the small-molecule development process, BBB permeability may be assessed at different stages by using several methods [20–22]. Computational models have been developed based on known drugs that act in the central nervous system (CNS) (called a "training set"). However, these models are limited by the small size of the training set and by the relatively narrow chemical space it covers [21]. In vivo assays can be conducted in both humans and animals using analysis of pure venous blood, microdialysis, and/or positron emission tomography (PET) imaging [20, 22]. However, these methods are generally low-throughput, labor intensive and costly. The use of in vitro assays, based on cell lines transfected with efflux transporters, was found to be a good complement to previously described methods [23]. It is also important to keep in mind that while BBB permeability is critical for drugs acting in the CNS, it is also an advantage to reduce the BBB permeability of drugs targeted outside of the nervous system to decrease potential CNS-mediated side effects. This is the case for loperamide, a therapeutic agent used to treat diarrhea [24]. Loperamide is a peripherally acting μ-opioid receptor agonist targeting receptors located in the large intestine. Because it does not cross the BBB, loperamide is devoid of analgesic properties usually observed with BBB-permeable μ-opioid agonists such as morphine.

Unfortunately, many drugs are marketed without providing doctors and patients information about BBB permeability and possible neurological side effects. An example of this situation is provided by the aromatase inhibitors (AIs), used to reduce the recurrence of estogen-responsive breast cancer, either in conjunction with or following treatment with tamoxifen [a selective estrogen receptor modulator (SERM) acting as an antagonist in breast tissue]. AIs work by inhibiting the aromatase (also called CYP19), the enzyme responsible for producing estrogen. Three AIs are currently the market: the non-steroidal agents anatrozole (Arimidex, AstraZeneca) and letrozole (Femara, Novartis), and the steroidal compound exemestane (Aromasin, Pfizer). Aromatase is present in several tissues including adipose tissue, uterus, bones and brain. While a reduction of estrogen levels in adipose tissue is critical to prevent the resurgence of breast cancer, studies also suggest that estrogen may be important for maintaining cognitive functions [25]. Hence inhibiting estrogen production in the brain may lead to cognitive decline. Strikingly, no information regarding cognitive function following chronic AI treatment is provided to the physicians or patients, even though these drugs were approved over 8 years ago. Studies regarding the possible effects of AIs on cognition in breast cancer patients have only been recently initiated, and, although preliminary results are beginning to come out, the numbers are still too small to determine whether there is an effect (reviewed in ref. [26]). If one or more of the AIs were known to be BBB impermeable, doctors and patients may be able to make a better informed decision regarding the treatment choices and quality of life.

Small molecules may be active at intracellular, extracellular, or both locations of targets and, as such, have traditionally been the platform of choice to act on enzymes and receptors. Moreover, depending on their physico-chemical properties, small-molecule drugs have been designed to be orally bioavailable, which greatly facilitates administration and patient compliance.

Biotherapeutics A biotherapeutic has thus far been considered to be a protein that works by mimicking the action of a naturally occurring substance. In many cases, the therapeutic was no more than the endogenous protein that was produced by a different method, for example, insulin produced synthetically using recombinant DNA technology as compared with purified insulin. Historically, protein therapeutics were isolated from human or animal sources, and their use carried risks such as variable efficacy, contamination by resident infectious agents, and immunological reactions. Most biological therapeutics are now manufactured using recombinant DNA technologies, allowing for a reliable and consistent product [27]. Immunogenic reactions to biotherapeutics are still a concern, but the added level of safety that is derived from a more chemically defined preparation justifies the approval as adverse reactions can be managed in most cases [28].

Recombinant protein therapeutics developed on the heels of the revolution in the area of molecular biological technologies. In 1972, the first article describing the use of restriction enzymes to cut two viral DNAs was published [29], and, by 1978, Genentech, the first company founded to develop biotechnologies, had expressed human recombinant insulin in *Escherichia coli* (*E. coli*) and produced about 20 ng of purified protein [30]. This joint venture with the pharmaceutical giant Lilly resulted in clinical trials beginning in 1980, and in 1982 human insulin manufactured by fermentation of *E. coli* became the first recombinant biotherapeutic to be approved by the FDA.

Over the years, recombinant therapeutic design has evolved and most protein therapeutics that are on the market are not exact duplicates of their endogenous human counterpart. For example, amino acid substitutions are sometimes included to improve pharmacokinetic properties [28]. In other cases, mutations may be induced to change a protein's function. Pegvisomant (Somavert, Pfizer) is a growth hormone receptor antagonist used to treat acromegaly (reviewed in ref. [31]). Substitution of 1 amino acid in the human growth hormone's 191 amino-acid-sequence is enough to turn this agonist into an antagonist. To create pegvisomant, eight other residues were mutated, increasing binding affinity to the receptor, and polyethylene glycol molecules were added to promote stability of the antagonist. Interestingly, even with modifications recombinant protein therapeutics are generally well tolerated and, for the most part, do not trigger immunological complications [28].

The use of antibodies as therapeutic agents has also emerged as a new technology. The success of immunization as a method to protect against infectious agents such as the measles and rabies virus stimulated thought that polyclonal antibodies isolated from human or animal serum could be used to combat toxins and venoms as well as to protect against infections. When this approach was tried, hypersensitive reactions were often triggered because the immune system recognized these polyclonal antibodies as foreign agents that needed to be removed. The advent of monoclonal antibody (mAb) production techniques in the 1970's, along with molecular biology, paved the way for the development and production of chimeric mAbs (murine Fv fragments linked to human IgG Fc fragment), humanized mAbs (human antibody except for the antigen-binding region, which is derived from mouse), and fully human mAbs (derived either from human B cells or from transgenic mice expressing human IgG). Although the immunogenicity of these mAbs is reduced, adverse reactions are still observed in some patients even with fully human antibodies, requiring coadministration of an immunosuppressive agent such as methotrexate (reviewed in ref. [32]). At the molecular level, antibodies may neutralize or antagonize the target, act as agonists, or deplete the target protein from the blood supply. The specific therapeutic action against a target is determined in part by the location of the epitope and by the constant region of the antibody. The ultimate pharmacological success of the therapeutic will require not only biological efficacy but a constant region that minimizes the induction of clearance, complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity or apoptosis.

The development of a biotherapeutic for rheumatoid arthritis (RA) treatment provides a good example of the challenges associated with mAb therapeutics [32]. RA is a chronic disease caused by a complex pathology that involves the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), and leads to the destruction of cartilage and bones of non-weight-bearing joints. A major difficulty associated with the use of mAbs is that animal models and ex vivo assays (such as whole-blood assays) offer limited predictability for human efficacy and adverse reactions. For example, at least eleven different antibodies against the T-cell surface antigen CD4 have been developed that are well tolerated and reverse autoimmunity in animal models. However, these same mAbs lacked clinical efficacy when tested in humans. The lack of equivalence between animal models, including non-human primates, and human subjects can sometimes have unexpectedly serious consequences. The activating, anti-CD28 mAb TGN1412 (CD28 SuperMAB, TeGenero Immuno) was shown to be extremely effective at reducing autoimmune reactions without apparent side-effects in both rodent and non-human primate models [33]. However, when TGN1412 was administered to healthy human subjects, all 6 volunteers developed multi-organ failure and extreme cytokine activation. It is unclear whether this reaction could have been otherwise predicted, but it emphasizes the differences between animals and humans when it comes to the pharmacological effects of antibody-based therapeutics.

In contrast to T-cell-directed agents, biological therapeutics targeting TNF-α have been very successful. FDA-approved agents include the anti-TNF-α chimeric mAb infliximab (Remicade, Centocor) and the humanized mAb adalimunab (Humira, Abbott). Both are well tolerated and produce immunogenic reactions in a very small fraction of patients, especially when taken with methotrexate. Another interesting agent is the fusion protein etanercept (Enbrel, Amgen/Wyeth), which is composed of the extracellular domain of the p75 TNF-α receptor attached to the hinge and constant domains 2 and 3 of the human IgG1. This biological therapeutic acts by binding TNF-α, reducing its concentration in blood and preventing its binding to the endogenous TNF-α receptors.

In addition to their traditional roles as immune modulators, antibodies can also be engineered to act as carriers designed to deliver a toxic "warhead" to specific target cells, such as in cancerous tissue. For this application, a specific mAb is conjugated to a toxin, small molecule, or radioisotope that is designed to selectively kill the targeted neoplastic cells [34]. This approach, in principle, allows the the accumulation of a high local concentration of active toxic compounds while minimizing side effects. For example, ^{131}I tositumomab (Bexxar, GlaxoSmithKline), which binds to CD20, is used for the

treatment of refractory non-Hodgkins lymphoma [35]. The CD20 antigen is present on normal and malignant B cells, including on 90% of B-cell non-Hodgkins lymphomas. Following a single infusion of ^{131}I tositumomab, 68% of patients saw improvement in their pathological and clinical profiles, including 33% who showed a complete response (Bexxar product information, GlaxoSmithKline).

The large-scale development of biotherapeutics is hindered by the limited options for drug delivery. Due to their large size and susceptibility to degradation in the gut, these agents must be administered via injections or by implantable control release devices. It is also currently impossible to use biotherapeutics for the treatment of disorders of the nervous system due to the fact they lack BBB and BNFB permeability. However this issue may be resolved as pre-clinical studies have been conducted using molecular Trojan horses; that is, fusion proteins between molecules that cross the BBB via receptor-mediated transport and BBB-impermeable agents (reviewed in ref. [36]). In those experiments, peptides such as the vasoactive intestinal peptide, and ribonucleic acid (RNA) interference (RNAi) against the epidermal growth factor (EGF) receptor were successfully delivered to the brain parenchyma. However, further studies will have to be conducted to determine the applicability of this method of biotherapeutic drug delivery to human nervous tissue.

Nucleic-Acid-Based Therapeutics The latest new category of therapeutic agents to emerge, in the 1990's, was based on nucleic acids. The early drug candidates in this group took advantage of antisense sequences as a method to specifically inhibit translation of a given gene product. Although the exact mechanism of antisense inhibition is not fully understood, it is believed that hybridization of a short, single-stranded DNA or RNA sequence complementary to an mRNA targets that mRNA for degradation and/or prevents the ribosome from binding to the mRNA, resulting in decreased levels of protein product. Administration of antisense could be used to treat a variety of conditions such as viral infections, cancers and disorders caused by a mutant protein exhibiting a gain-of-function. However, there are also numerous challenges associated with these therapeutic agents including the short half-life of oligonuclotides in biological fluids, their cellular uptake, and the toxicity they may induce [37]. Chemically modified bases can be used to increase stability of the antisense oligonucleotides in vivo. The most common is the phosphorothioate deoxynucleotide, which increases serum half-life from 1 hour for standard oligonucleotide to 9–10 hours for phosphorothioate-containing oligonucleotides. Cellular uptake can be improved by using delivery systems composed of lipids, polymers or nanoparticles. An alternative strategy is to conjugate the antisense oligonucleotide to an antibody or ligand recognizing a receptor on the surface of the cell, achieving targeted delivery. Toxicity is often the result of an off-target effect(s) and needs to be assessed on a case-by-case basis [39].

The first and only FDA-approved antisense oligonucleotide to date is fomivirsen (Vitravene, ISIS Pharmaceutical and Novartis). This therapeutic agent received approval in 1998 to treat cytomegalovirus-induced retinitis in AIDS (acquired immunodeficiency syndrome) patients (reviewed in ref. [38]). Fomivirsen is composed of phosphorothioate deoxynucleotides and is administered intravitreally. However, although this medication answered a specific medical need, the small number of patients and low sales lead Novartis to discontinue its production. Several other antisense therapies have entered clinical trials, but none have demonstrated sufficient efficacy to obtain FDA approval [37].

RNA interference (RNAi) is a method endogenously used by a wide variety of organisms to target mRNA molecules for degradation [40–42]. Unlike antisense-mediated inhibition, the mechanism by which RNAi works is well understood, and several putative therapeutic agents at different stages of development make use of this technology [43, 44]. RNAi can be achieved using micro-RNA (miRNA), small interfering RNA (siRNA) or short hairpin RNA (shRNA). miRNAs are endogenously encoded single-stranded RNAs that interact with the 3′ untranslated region of mRNAs. Since they do not require perfect sequence homology, miRNAs can interact with 100–200 genes, some of which may be involved in the same signaling pathway [43]. Inhibition of a given mRNA by miRNAs is not complete, but additive effects may be observed if several transcripts in the same pathway are targeted by one miRNA. Endogenous miRNAs, such as miR-146, miR-155 and miR181a, are involved in the development and regulation of the immune system and are altered in chronic inflammatory diseases [45]. In addition, miRNA changes have been reported to correlate with the progression and prognosis of certain cancers [43]. These observations suggest that miRNAs may be an interesting therapeutic target.

The other two types of RNAi tools, siRNAs and shRNAs, are exogenously applied to modify gene expression [46]. siRNAs are short (19–23 nucleotide) double stranded RNAs with a perfect complementarity to their target mRNA. Once they enter the cell, they are taken-up by the RNA-induced silencing complex (RISC) which unwinds the siRNA and degrades the sense strand. The antisense strand is then used as a template to identify the target mRNA, which is then degraded by Argonaute-2. A good siRNA can mediate >90% inhibition of its target. A number of siRNAs are

currently in clinical trials [43]. While naked siRNAs may be appropriate for targeting easily accessible tissues such as the eye or the lung, they are not stable enough to be delivered via systemic circulation. For deeper tissues such as the kidney, chemical modifications and/or delivery systems are used [43]. The most advanced siRNA, currently in phase III clinical trials, is bevasiranib (Opko) against vascular endothelial growth factor. It is a naked siRNA administered by intravitreal injection for wet age-related macular degeneration. AKIi-5 (Quark/Silence) is a chemically modified siRNA that targets p53 and that is delivered intravenously. It recently began phase I trials for acute renal failure.

As their name indicates, shRNAs are short, single stranded RNA sequences that form a hairpin. In the cytoplasm, this hairpin is cleaved by Dicer, leading to an siRNA that can interact with the RISC as described above. The advantage of shRNAs is that they can be encoded on a viral vector, which can be engineered for tissue-specific and/or controlled delivery (reviewed in ref. [47]). A variety of vectors have been used successfully in animal models, including adenoviruses, adeno-associated viruses (AAVs), and lentiviruses. Two shRNAs recently entered clinical trials, targeting hepatitis B infections (Nucleonics/Novosom) and AIDS-related lymphoma (Benitec).

RNAi methods are not without risks and challenges. The most common issue observed in animal models has been the risk of saturation of the endogenous RNAi machinery. For example, in mice receiving large doses of one of 49 different AAVs encoding for shRNAs against 6 different genes, 36 constructs led to liver toxicity, including 23 which resulted in death [40]. This was shown to be due to saturation of Exportin-5, a transporter for precursors of siRNAs and miRNAs. This serious problem can be avoided by using naked siRNAs (mature siRNA enter the RNAi machinery downstream from Exportin-5) or by using a lower titer of virus. Viruses engineered to offer controlled expression of their transcript may also be useful, representing a significant opportunity for the development of new therapeutics.

Using RNAi may also cause off-target effects and/or trigger an interferon-mediated response. Both of these can be reduced by chemically modifying the siRNA and by avoiding specific sequences, which are known to be immunogenic. Finally, resistance to a particular RNAi may develop as a single base-pair mutation is enough to disrupt the interaction between an siRNA and its target. This may be avoided by engineering a vector with a few different shRNAs against the same target.

Portrait of Drug Approval in Recent Years Most new drugs authorized by the United-States Food and Drug Administration (FDA) are classified as small molecules, whereas biotherapeutics account for 5–29% of the yearly approvals (Fig 1.2). However, despite technical advances and increased spending in research and development by pharmaceutical companies [48], the number of drugs approved has been declining since 1996. This effect is particularly noticeable for small molecule

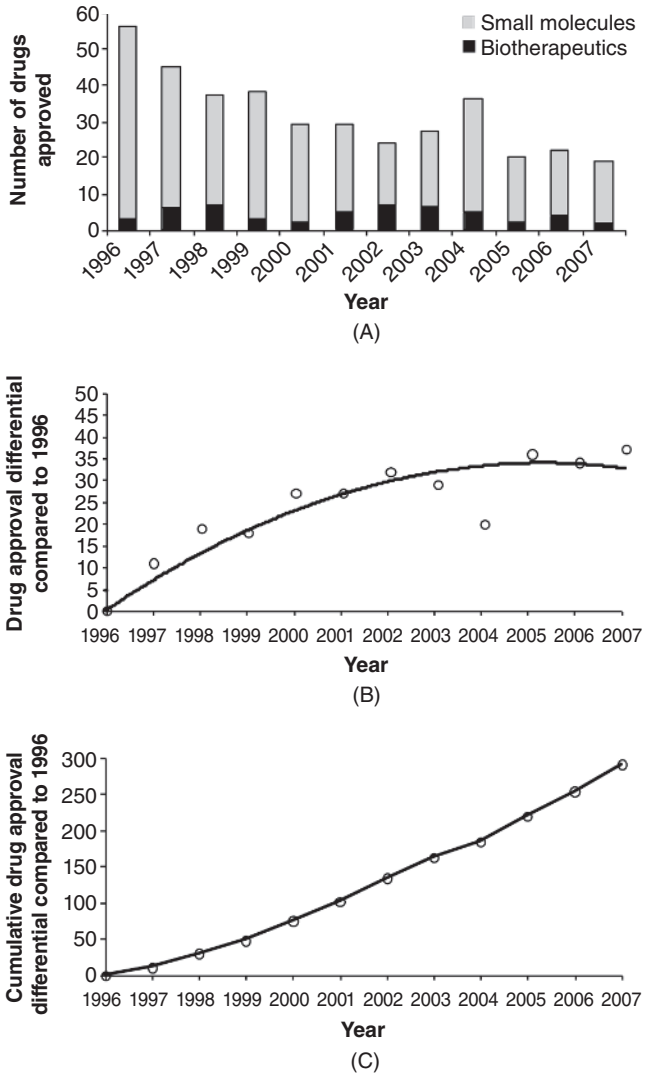

Figure 1.2 Drug approval by the Food and Drug Administration (FDA) since 1996. (A) Number of new drugs approved per year by category. Small molecules (gray bars) require the submission of a new drug application (NDA), while biotherapeutics (black bars) are reviewed under the form of a biological licence application (BLA) (Data source: www.fda.org.) (B) The drug approval differential, as compared to the number of drugs approved in 1996, reveals that there has been a progressive decline in approvals in the past ten years. In the last few years however, rate of decline seems to be leveling off. (C) Despite stabilization in the number of new drug approvals, the cumulative drug approval differential suggest that if approvals had been maintained to the level of 1996, we would have nearly 300 additional new drugs on the market today.

therapeutics. There are many reasons for this trend, including increased requirements for safety, reduced side-effects, and a litiginous system of law [39, 49]. Given the current risks and elevated cost of developing a new drug, pharmaceutical and biotech companies often turn to repurposing, the identification of new indications for existing drugs, or to reformulation, such as developing continuous release or combination therapies, to increase their market share [49, 50].

If the outlook for new drug discovery at large is declining, the glacier trail leading to breakthrough neurotherapeutic agents is even more daunting and treacherous. According to the World Health Organization [51], 40 million people worldwide are affected by epilepsy, 24 million suffer from Alzheimer's disease or other forms of dementia, 62 million are diagnosed with cerebrovascular diseases, and 326 million suffer from migraine. However, despite the high prevalence of these disorders, only a few drugs with neurological indications are approved each year (Fig. 1.3). Clearly, therapeutic agents targeting neurological conditions must cross the BBB, but it is estimated that 98% of putative neurotherapeutics fail due to lack of BBB permeability [52]. In addition, candidates often fail during clinical trials due to poor efficacy or safety concerns. As expected, CNS-directed agents tend to cause CNS-mediated side effects such as seizures, dizziness and nausea. Moreover, the lack of validated biomarkers makes it difficult to assess whether the drug reaches concentrations that are sufficiently high at the target to be efficacious [53]. In 2006 alone, 11 drug programs targeting 7 neurological disorders were halted during clinical testing [54]. In the current situation, there is a dire need for new therapeutics directed towards even the most common of neurological conditions. It is sobering to note that with all of its undesirable side effects and adverse reactions morphine, and its derivative opiate receptor agonists, still provide the mainstay of analgesics.

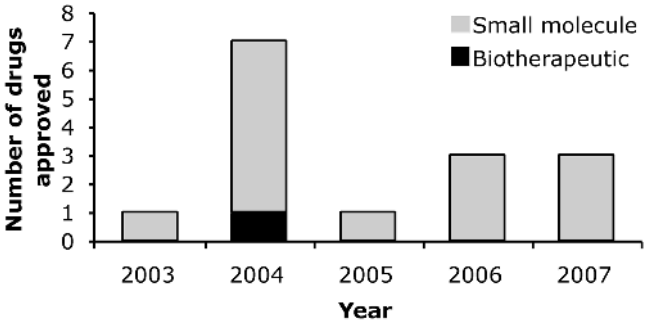

Figure 1.3 Number of new drugs approved by the FDA from 2003 to 2007 with a primary indication for a neurological disorder or neuropathic pain. Small molecules are indicated by gray bars and biotherapeutics by by black bars. The only biotherapeutic recently approved is natalizumab (Tysabri, Biogen/Elan), an anti-α4 integrin monoclonal antibody used to treat relapsing multiple sclerosis (Data source: www.fda.org.)

1.2.2 Need for New Therapeutic Agents

Over the last century, there have been major advances in our understanding of pharmacology, biology of disease, and in methods for the discovery of therapeutic agents toward a wide range of maladies. Indeed, the discovery of the "wonder drugs", the tricyclic antidepressants, phenothiazine antipsychotics, and benzodiazepine anxiolytics, completely changed the way society thought about mentally ill: from the notion of insanity and incarceration to the concept of neurological disorder and outpatient treatment. However, there is a great need for continued research and development to improve existing therapeutics, develop new classes of pharmacotherapies for diseases where the current treatments may not work in some or even most patients, and to establish new treatments for conditions where none are available. Given the decrease in new drug approvals in the last several years, it is important to emphasize that there is a crucial need for more new therapeutics to be developed and submitted for review. In order to improve its productivity, not only must the pharmaceutical industry rethink its drug discovery strategies but the federal government and international world health agencies must work to provide an optimal setting for the high risk high stakes business of drug discovery and development to thrive.

Improving on Currently Existing Therapeutics It is easy to imagine the properties of the ideal drug: orally bioavailable, once a day treatment, with no side effects, and completely effective in all patients. Improving on existing therapeutics is an important and valuable method to generate new therapeutics. This strategy mitigates the risks associated with first-in-class therapeutic agents as the chemical genres and target are often well-validated, and certain aspects associated with modulation of the target in humans have been explored. Among the improvements that are generally feasible are enhancements in efficacy, and/or potency, and/or specificity, all aimed at enhancing desired as compared with undesired pharmacological effects via altered pharmacodynamic and/or pharmacokinetic properties. Incremental improvements on the method or frequency of delivery can also provide significant benefits to patients and thus justify the allocution of limited resources to such objectives.

However, this is not a safe haven for the pharmacologist as any modification can cause deleterious side effects even if the discovery effort focused on the creation of a new therapeutic agent acting on the same

target as the approved drug. Novelty is also a key aspect of new drug design, whether small molecule or biotherapeutic, as patent protection is an essential element to commercialization. While this driver is often bemoaned, it serves a useful purpose in rewarding pharmaceutical companies that bring breakthrough science to the benefit of medicine via the market place.

Finding New Targets for Conditions Where Therapeutic Agents Already Exist An exciting and challenging direction is to discover a new therapeutic agent that acts at a novel target that improves the treatment relative to a medication that already exists. The target can be selected from within a pathway that has already been exploited or in a different pathway that is also important for the disease biology. In addition to resolving intellectual property issues identified with the previous strategy, this approach offers a number of advantages. For example, a drug acting at a different target may be effective in non-responders to existing therapeutics due to a number of factors, such as tolerance, heterogeneity of disease pathology, or variations in genetics among populations. A therapeutic agent with a new mechanism of action is also likely to have a different side effect profile, which may be better tolerated. However, working on a new target brings risks that are usually not a factor when working with a known target. For example, choosing a target which is involved in multiple biological pathways or whose biological role(s) is(are) poorly understood may lead to unanticipated adverse events, such as previously described for the super-agonist anti-CD28 mAb TGN1412 [33], or the breakthrough discovery of the selective cyclooxygenase-2 (COX-2) enzyme inhibitor rofecoxib (Vioxx, Merck). It is, therefore, sobering to realize that the particular target must be chosen carefully and thoroughly validated to ensure that it is both implicated in the therapeutic pathway and unlikely to produce serious adverse effects when its activity is modulated. In this regard nature is unforgiving and while patent law rewards novelty, the best science may not always be rewarded. As an example, while Merck scientists discovered a selective COX-2 inhibitor rofecoxib, a number of adverse events that would result from selective inhibition of this enzyme were not anticipated. Inhibition of the COX-1 enzyme was deemed responsible for the gastro-intestinal bleeds observed following chronic treatment with non-steroidal anti-inflammatory drugs (NSAIDs). Pfizer developed the less selective and therefore seemingly less innovative drug celecoxib (Celebrex), which inhibits mainly COX-2 at a therapeutic dose with minimal effects on COX-1 [55]. Although mechanistically similar, rofecoxib was voluntarily withdrawn yet celecoxib has remained a specialty pharmaceutical with a black box warning. This teaches us that the objective of developing a highly potent selective inhibitor of one of two enzymes in a pathway firmly implicated in a physiological process is sound but the side effects that result from involvement of a particular target in other patho-physiological processes can surface as well.

Combination therapy using two or more classes of therapeutic agents to treat a disorder can also provide substantial benefits with reduced risks. This approach has helped with the management of human immunodeficiency virus 1 (HIV-1) infections, for which there are currently six different classes of drugs available. According to the U.S. Department of Health and Human Services, initial therapy with two or three antiviral agents is most effective at reducing HIV-1 load since infected patients often present with a small proportion of viruses harboring drug-resistant mutations [56]. Upon resurgence of the viral load, a new drug combination can be used to suppress infection by a resistant virus strain.

In addition to delaying the appearance of drug-resistant viruses or cancer cells, combination therapy may also be useful to increase the efficacy of a treatment by co-administering two drugs which are not completely effective when given alone. This strategy is often use for the treatment of hypertension, where single agents may not sufficiently lower the patient's blood pressure [57]. Another possibility is to combine two therapeutics at a sub-optimal dose to reduce side effects and achieve increased efficacy. One example is the combined use of acetaminophen with an opioid receptor agonist such as oxycodone. Acetaminophen reduces the dose of the narcotic analgesic required and thus reduces the adverse side effects, limitations, and liabilities for both. However, de novo side effects or undesirable events may arise. These may result from interactions of the different drugs with liver metabolizing enzymes, competition for transport mechanisms, or biological interactions between the different targets and pathways modulated. For example, in prescription drug narcotic addiction, addicts learn that pulverizing controlled-release oxycontin tablets provides a rush by circumventing the controlled-release formulation. Consumption of large amounts of acetaminophen/oxycodone can also result in chronic overdose of acetaminophen, possibly resulting in severe kidney damage.

Finding New Therapies for Untreated Conditions Perhaps the greatest challenge, and the greatest need, in drug discovery resides in finding therapeutic agents against disorders for which no treatment exists. Factors influencing the decision to launch a new drug discovery program include unmet medical need, market size, the ability to diversify the company's intellectual property

portfolio, the availability of required technical expertise, the prospects for upcoming competitors, and the anticipated level of difficulty with the new drug approval process [27]. Although pharmaceutical companies generally focus on a disease that affects a significant portion of the population, there has been a trend in the United States for smaller biotech companies to work on orphan drugs, designed to treat rare disorders affecting less than 200,000 Americans [49]. In addition, for therapeutic agents that treat serious medical conditions where there is an unmet medical need, a priority application can be filed with the FDA, which accelerates the approval process and reduces the total time to market. In recent years, the proportion of priority applications approved by the FDA has risen in comparison with standard approvals (see www.fda.gov), suggesting that this avenue may be advantageous for both medical and commercial reasons.

The identification and validation of a target remains a major challenge with developing a drug for an untreated condition. This is especially true if the disease biology is poorly understood. While the recent emphasis by the pharmaceutical industry has been on identifying a single target and creating a highly potent and specific drug, examples taken from older "dirty" drugs and combination therapies show that drugs acting on multiple subtypes of a single target group or on multiple targets can be highly beneficial. One example is the nonselective benzodiazepine positive modulator diazepam which acts on at least twelve different γ-aminobutyric acid ($GABA_A$) receptor subtypes containing the γ_2 subunit. Another example is the double reuptake inhibitor venlafaxine (Effexor, Wyeth), which inhibits both norepinephrine and serotonine transporters.

1.3 APPROACHES FOR DRUG DISCOVERY

Historically, drug discovery began with the empirical observation of a therapeutic effect, often by random testing in animals, which was then confirmed and further refined using bioassays and preclinical models (Fig 1.4).

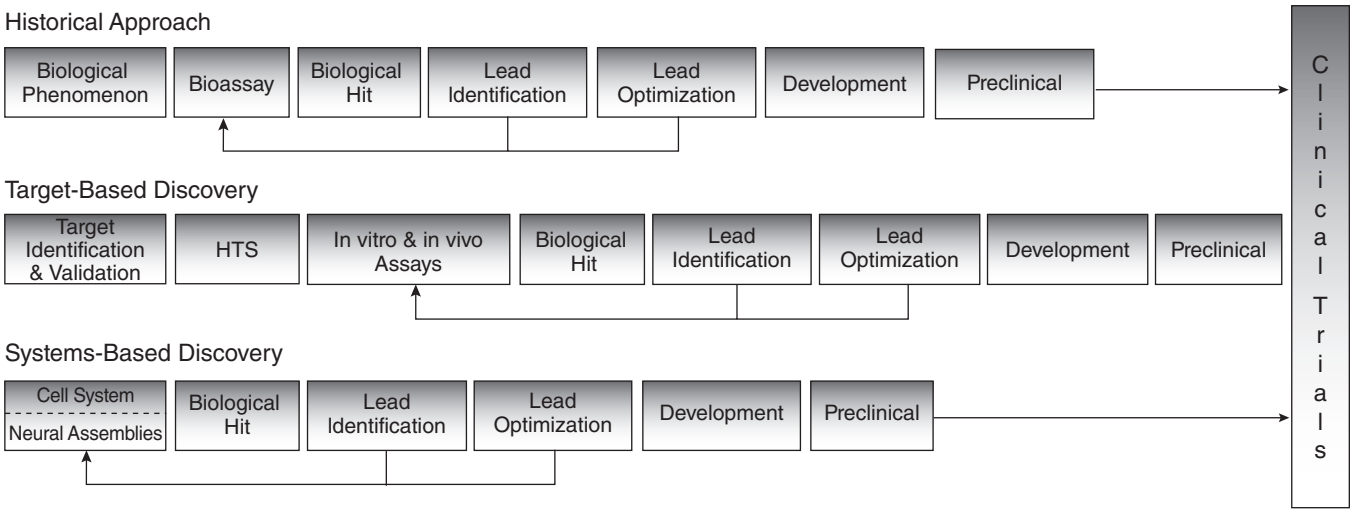

Figure 1.4 Drug discovery pipeline. The discovery of new a therapeutic can be initiated in a number of ways as illustrated here by the historical approach, target-based discovery (TBD) and systems-based discovery (SBD). Although the first step (first box for each method) differs, the lasts steps of the process leading to optimization and development of the compound, pre-clinical, and clinical trials are fairly similar. In the case of SBD, the nature of the initial screening system dependent on the disease studied. For example, while cancer and inflammation can be modeled accurately using a collection of human cells, neurological disorders may require a more complex system such as an *in vivo* recording model in order to obtain data from neural assemblies. Neural assemblies are units of neurons whose activity, when taken together, contributes to the observed behavior. Interestingly, although the approach proposed for systems-based drug discovery seems to be similar to the historical approach, our improved understanding of the disease biology today facilitates the selection of an appropriate model for screening compounds, eliminating the need for a trial-and-error approach and increasing our chances for success. It is assumed tha for SBD, the input of compounds would not necessarily involve HTS but could begin with high value focused chemical libraries.

Today, in the postgenomic era, there has been a change in paradigm. Drug discovery is now usually initiated by the identification and validation of a target, even before any putative therapeutic is tested. While this was an effective strategy in the late 1980's and early 1990's, the trend towards decreasing new drug approvals during the past decade raises questions about the long-term effectiveness of this approach. In addition, to overcome challenges associated with the increased scrutiny from regulatory authorities and the ever-rising cost of development for new therapeutic agents, the industry may consider revising its functional model for drug discovery, by transitioning from a target-based approach to a systems-based or combined approach.

In the sections below, we review current drug discovery strategies and suggest a number of opportunities for advances based upon existing needs for improvement.

1.3.1 Target-Based Discovery (TBD)

Target-based discovery is the most widely used strategy by pharmaceutical and biotech companies. Although specific details may vary, the general framework of TBD is usually similar across companies (Fig 1.4). The first step is to identify a target that is important to the onset or progression of disease pathology [58]. As part of this process, the "druggability" of a putative target, that is, whether and how such a target could be modulated by a therapeutic agent, is of key strategic importance [59]. In addition, financial considerations, public perception of a disease, and governmental health policies may also affect the target selection process [60]. Previously validated targets are an obvious choice, as discussed above, but information regarding novel targets may be obtained from various sources. In some cases, human genetic associations identify a chromosomal area or specific gene that is linked to the disease and further associated based upon a valid animal model [58]. If no suitable target can be identified from in vivo studies, in vitro methods including activity assays and gene or protein expression studies may provide useful information. Alternatively, in silico methods such as bioinformatics (genomics, proteomics, transcriptomics) and pathway analysis may allow to rapidly identify potential targets, but these will require additional validation [61]. It is important to note that results from all the above-mentioned methods may also be found in the literature. Although targets identified from the literature are not technically novel, because the knowledge is publicly available, they can present a lower risk profile if they emerge from high-quality data.

The next step in TBD is to validate the targets identified. This is a crucial step in that it provides the foundation for the entire drug discovery program. Failure to properly validate a target may cause the program to fail at later stages when much time and financial ressources have already been invested. To establish a validated target, several criteria must be fulfilled [58]. When comparing patients affected by the disease and healthy controls, a good target may have a different expression level or activity level. However, interpretation of data such as a change in protein levels or activity may reflect either the cause of the disease or the consequence of the disease. Changes could also result as an adaptive self curative process. In the later case, inhibition of a molecule involved in a negative feedback pathway may worsen the disease. As part of the validation process, the expression pattern of the target molecule must be assessed and localized in a tissue that is relevant to the pathology. Clearly, the ultimate step in target validation is to demonstrate that modulating the target in human patients improves their condition. This is a demanding task but of great importance. New technologies and/or strategic approaches that advance the validation of the target function in human disease will be on the cutting edge for years to come.

As its name suggests, TBD is based on finding drug candidates that act specifically at the chosen target. In the pre-exploratory phase of drug discovery, the assays that are going to be used to test drug candidates are developed, and the specific assays chosen depend upon the type of target. For example, enzyme targets can be tested conveniently in isolated protein assays [62]. However, to find a drug modulating a G-protein-coupled receptor (GPCR), a functional screen in a cell-based assay may be more appropriate. This approach could employ a focused chemical library of a small number of compounds and lower throughput high value screens, such as high throughput electrophysiology [63], or high throughput screening (HTS) of large scale compound libraries.

For the HTS approach, the first step precludes washes and requires an output that can be measured easily [64]. Secondary assays of increasing order of complexity are conducted following the HTS screening. These assays utilize automated benchtop technologies, can be more detailed, but should still have a throughput that is high enough to screen thousands of hits from the HTS screen. The secondary assay phase can employ a cell-based assay, if not already carried-out in the primary assay, and includes selectivity screens. Assessing selectivity early-on is critical as off-target effects may induce side effects or adverse events in pre-clinical studies.

Following in vitro assays, short-term in vivo assays are performed. These assays can usually be carried out on the time-scale of hours to 1 or 2 days and require less compound. Although they may not be a good predictor of efficacy in the disease model, they allow

a determination as to whether the compounds can have some effect when introduced into an animal. Pharmacokinetic studies establish the dosage and route of administration to be employed in the long-term in vivo studies using animal models of disease. At the end of the pre-exploratory phase, an established screening tree beginning with target-based screening and ending with selective pre-clinical animal models of disease is fully implemented.

Once the assays to test the activity of compounds at the target have been established, the TBD exploratory phase can be initiated. The objective of this phase is to identify hits from a library of compounds, and then from those hits to generate one or a few lead families of compounds. Following HTS, thousands of potential hits may be identified [64]. If structural information is available on the target or on a known ligand of the target, pre-screening of the library using structure-based computer modeling may help decrease the total number of hits and increase hit quality [61, 64]. Compounds identified by HTS then have to be re-screened and/or counter-screened to confirm their activity and establish a basic dose–response curve. After confirmation, hits are subjected to the secondary assays devised during the pre-exploratory phase. Following each assay, compounds are ranked and structure–activity relationships (SAR) and structure–property relationships (SPR) emerge. These relationships help to better understand how the compounds work and how they can be improved using chemical modifications [65]. A few hits are generally selected to undergo iterative rounds of chemical modification and testing in secondary assays. At this point, one or a few lead series are typically chosen to move on to the next step.

For each lead series in the discovery phase, the SAR and SPR would be further characterized, pharmacokinetic/pharmacodynamics (PK/PD) studies performed, and candidates tested in in vivo models. It is interesting to note that for TBD this is the first phase where chemical entities are actually introduced in animals. As a result, compound production must be scaled-up to achieve a medium scale [66]. PK studies are important to establish the time-course for drug absorption, distribution, metabolism and excretion [67, 68]. As part of the metabolism studies, possible interactions between the drug and the hepatic cytochrome P450 (CYP) family of enzymes are tested. Drug-mediated inhibition of a CYP enzyme that is responsible for the metabolism of the compound studied or of other commonly used therapeutic agents may lead to severe side effects or to drug–drug interactions [68]. Given the possible severity of the consequences of CYP inhibition, such occurrence would be carefully evaluated. PD studies, on the other hand, relate the pharmacological and toxic effects observed upon administration of a drug to the concentration of its active metabolite in the plasma (or other tissue of interest).

Throughout this process, chemical modifications are still being performed to improve the compounds' properties and to address any potential issues that could result in failure to identify a lead compound. The information obtained from the PK/PD studies is critical for the subsequent in vivo studies as the dosage and dosing frequency selected need to provide an adequate drug concentration for the appropriate duration to obtain a therapeutic effect with minimal toxicity [69]. Animal studies begin with the short-term models devised during the pre-exploratory phase, which have a faster readout and require less compound. Then, the more promising candidates are tested in the long-term disease models. While in vivo testing is present throughout the discovery phase, determination of the efficacy of a compound in a disease model is not done until the later part of this stage and, although chemical optimization is ongoing, there is a risk that the lead(s) obtained may fail and be unsalvageable.

When a lead compound or compounds is/are identified, further testing in non-human primate models including PK, PD, proof-of-concept, and efficacy in a disease model are carried out. This last testing phase allows to obtain information which may be more indicative of the drug's effect in humans, although some unpredicted events may still occur later on in the clinical trials. If a good TBD lead candidate is obtained, drug development can begin.

As described, TBD is a lengthy process with a general progression from the simplest, most reductionist models towards the more complex systems. In parallel, iterative rounds of chemical modifications are performed to establish SAR and SPR in an attempt to improve the compound properties and resolve problems that may arise. While TBD has been successful in identifying new drugs against previously validated targets (i.e. modulated by a known drug), generation of therapeutic agents acting at novel targets has been disappointing [70]. This can be explained in part by the fact that although TBD leads to compounds whose mechanism of action and contact sites with the target are reasonably well-understood, it is also prone to late-stage failures as testing in more relevant in vivo models is not done until the end of the discovery process. This creates an unfortunate situation where, by the time an advanced compound is rejected, several years of discovery and huge opportunity costs have already been invested in the project without any return.

1.3.2 Systems-Based Discovery (SBD)

As the limitations of TBD described above become evident, an alternative view of drug discovery is emerg-

ing. Systems-based discovery (SBD) methods are based on the use of complex systems that model the disease to look for potential therapeutic compounds, instead of focusing on simpler assays that test drug interactions with a single target (Fig 1.4). While SBD might in some way seem to be a return to the traditional approach to drug discovery, recent technologies allow for the automation of high content assays, with a concomitant increase in throughput. However, advances in computational modeling facilitate the identification of pathways and mechanisms important to the disease biology [70, 71].

A range of systems could be suitable to use as the starting point for SBD. Human cell systems offer many advantages and provide a practical approach to SBD [70]. The complexity of the pathways involved in a disease may be modeled better using a combination of human cell types instead of a cell line. Rather simple modifications can also be made to a human cell system to reflect different physiological or pathological states, further increasing the chances of uncovering therapeutic properties for the drugs tested. For example, a model system for studying inflammatory diseases could be composed of endothelial cells and blood mononuclear cells, stimulated using a combination of cytokines [70]. A disadvantage of such a system, however, is that it may be difficult to obtain the correct combination of primary human cells that will meaningfully reflect the biology of the disease. Some conditions, such as psychiatric disorders, may not be amenable to cellular-level modeling while others, such as these regulated by organism-wide hormonal signaling, may only be modeled crudely by a human cell system. However, new stem cell technologies may remedy this deficiency by providing an endless supply of undifferentiated tumor cells and tissue components.

Another advantage of SBD compared to TBD is that drug screening is performed using a marker of disease progression/regression so there is no procedural requirement to identify and validate a target which, in principle, could reduce discovery costs by allowing only higher value molecules to be identified as leads. Moreover, although target validation in animal models of disease is widely accepted, a given target overexpresed in a cellular or non-cellular system may select for molecules that work in an animal model but are not as relevant to the disease pathology in humans or target modulation may cause unexpected adverse effects. SBD screens compounds against an unknown number of targets but would theoretically generate relatively advanced hits.

Following system-based screening, further testing is conducted and the promising compounds are optimized. As previously described for TBD, these assays include PK and PD characterization as well as short- and long-term animal models. At this stage, a lead compound may be obtained. If desired, target deconvolution strategies can then be applied to identify the target or targets that is/are modulated by the lead compound (specific examples of deconvolution techniques are reviewed in ref. [72]). However, these methods depend upon a physical interaction between the lead compound and one or more targets, with varying affinities and efficacies. Target deconvolution can be straightforward to intractable and should be considered optional as clinical trials can be initiated if safety and efficacy have been demonstrated in cell-based and animal models of the disease [70].

A more complex alternative to the use of human cell systems is to generate system response profiles [73]. These can be obtained by subjecting samples such as body fluids or biopsy samples to various analyses (gene array, mass spectrometry, NMR spectrometry, immunoassays, etc) to obtain levels of gene transcripts, proteins, or metabolites. System response profiles can then be compared across healthy and sick individuals or before and after administration of a therapeutic compound, for example. By collecting samples at different time points, an analysis of the system's dynamics can also be performed. This extracts information about disease progression and/or provides a better understanding of the transient effects of a therapeutic agent. System response profiles may help identify sub-groups of patients with a given diagnosis that are expressing different biochemical markers.

For a systems pharmacology assessment, such a classification may also provide biomarkers for drug discovery and help predict which patients are likely to respond to a given treatment. This application alone could help increase the success of clinical trials, especially for neurological disorders where the lack of validated biomarkers has been cited as a cause for the high rate of failures, due to possible improper patient classification and difficulties in assessing the suitable amount of drug to use [53]. In a different application, system response profiles may be useful in assessing the validity of an animal or cell-based model of the disease [74]. By comparing the system response profiles of the disease model to that of human patients, it may be possible to determine which pathways hold predictive validity and are more likely yield a successful therapeutic agent. Furthermore, different models may be better predictors for different subgroups of the patient population.

Golub and colleagues developed a similar system, called the Connectivity Map, using data collected from treating human cells with a variety of biologically active compounds [75]. This publicly accessible database can be searched for information regarding the activation of certain genes following the treatment of cultured human cells with drugs. By comparing different profiles a new biological function for a known compound can

be identified. Gene expression-based high-throughput screening (GE-HTS) has been used to screen for inhibitors of platelet-derived growth factor receptor (PDGFR) signaling [76]. PDGFR signaling leads to activation of the extracellular regulated kinase (ERK) pathway, which is known to be up-regulated in a number of cancers. In human TIP5 fibroblast cells treated with PGDF with or without an ERK inhibitor and subjected to microarray, a number of regulated genes correlated with ERK activation were selected as markers for ERK inhibitor activity. Application of GE-HTS to a chemical library led to the identification of aurintricarboxylic acid, which inhibits PGDFR phosphorylation, preventing downstream activation of ERK. GE-HTS does not require a detailed knowledge of the signaling transduction cascade and captures molecules that act either upstream or downstream of the known kinase.

When applied during clinical trials, system response profiles may also generate useful information and potentially rescue drug candidates that would otherwise fail [73]. For example, the system response profiles of drugs with limited efficacy could be used to predict useful therapeutic combinations, either with a previously marketed agent or with another low-efficacy drug. System response profiles may also help to better understand mechanisms by which drugs work, including potential off-target effects. These unknown mechanisms can either enhance therapeutic effects or cause side effects. Knowing which disease pathways are targeted by a given treatment may also expose unexploited areas of the disease profile, including potential new drug targets.

Despite these limitations and given their potential benefits, system response profiles could fulfill the need for the discovery of new therapeutic agents by delivering higher value lead candidates earlier in the discovery process.

1.3.3 Balancing TBD and SBD

TBD and SBD offer very different strategies, as illustrated by the dissimilarities in their workflow pipelines (Fig 1.4). In TBD, the first discovery milestone identifies and validates a drug target. By contrast, SBD begins with the development of a systems-level assay representative of the disease and then proceeds with testing a library of compounds to identify hits. With TBD, assay development and hit generation occurs later in the process, following target identification and validation. SBD generates hits at an earlier stage and, at least theoretically, selects compounds that have better druglike properties. Whereas TBD uses simple and usually cell-free assays for HTS, SBD is generally carried-out using an intact cell assay on a system ideally composed of human cells. The quality of the hits obtained plays a major role in determining how much effort will have to be devoted to lead identification/optimization. Another major difference between the two strategies resides in the fact that in TBD, compounds are tested in cell-based systems during the discovery stage, which occurs much later in the process as compared with SBD, which begins with a cell-based system or tissue. In concept, compounds identified by HTS in TBD fail at a much later stage than those from SBD. Given these differences, one may think that, on average, SBD would generate more higher quality leads in a shorter time span. However, this may be counter balanced by the fact that additional efforts will have to be devoted to identify the target(s) modulated by the SBD lead compound. This last step is useful from the point of view of understanding how a given compound works, but it is not required to fully understand the mechanism of action of a drug in order to obtain permission to test it in humans. The prerequisites for a compound to enter clinical trials are demonstrated efficacy and safety in animal models of disease. As can be seen from this discussion, the development of a systems-based approach to drug discovery is in its infancy and represents an emerging need for new technological approaches.

Once an optimized lead compound is obtained, the remainder of the process to obtain a new therapeutic agent is similar between TBD and SBD, even though outcomes may be different. In both cases, drug candidates need to be tested in animal models of the disease. In addition to being ready for the preclinical phase faster, leads obtained via SBD may act on several targets. In contrast, leads from TBD are, in principle, selected to be effective at and selective for a single molecular target, although side effects exerted via actions at multiple unanticipated mechanisms is common. While modulation of a single target may be sufficient to treat a disease, cellular pathways are often resistant to perturbations [77], and drugs acting at several targets may be more successful. For the development phase, TBD and SBD converge. At this post-discovery stage, there are major differences between the preparation of small molecules and biotherapeutics. The development phase is the last stage before the drug is tested in humans. It consists in establishing manufacturing conditions to produce the compound and conducting any additional pre-clinical studies that may be needed. It is also in this phase that the clinical trials are planned. This requires determining the population to be tested, establishing protocols and planning for centers to conduct the trials. When this is done, an Investigational New Drug Application can be filled with the regulatory agency. This document details the findings obtained in in vitro and in vivo studies, with particular emphasis on

safety and efficacy, and the plans for clinical trials. If there are no concerns on the part of the regulatory agency, clinical trials are allowed to begin.

Administration of a test substance into humans is a highly regulated process whose details are beyond the scope of this chapter. However, to summarize the process, clinical trials are divided into three phases. Participants in each phase are required to sign an informed consent form detailing the potential risks and benefits associated with their participation in the trial. In phase I clinical trials, a small number of healthy subject are recruited to test the safety of the investigational new drug. The first PK/PD studies are also conducted during this stage. If no adverse events are reported, the study then proceeds with phase II. At this stage, varying doses of the drug are tested in an intermediate number of patients afflicted by the condition studied. These studies may also be conducted with a group of control patients receiving a placebo (with or without the standard approved treatment). The objective of phase II trials is to establish an efficacious dose and dosing regimen for the drug. In phase III, the drug is tested in a large number of patients across different geographical areas in a double-blind, placebo-controlled trial. The objective of this phase is to establish efficacy in a demographically diverse patient population. The large number of subject recruited may also help in identifying unforseen events occurring in only a small fraction of the population, which may have been missed in phases I and II due to the small sample size. Following the completion of the phase III studies and if the drug is deemed safe and efficacious, a New Drug Application (for a small molecule) or a Biological License Application (for a biotherapeutic) is filed with the regulatory agency to obtain the permission to commercialize the new therapeutic agent. As compounds enter clinical trials, it is thought that the SBD pipeline would be more successful on average due to early selection for activity in complex animal and/or human cell systems leading to the optimization of low toxicity hits targeting several members of a pathway or even different pathways involved in the disease.

1.3.4 Brief Look at SBD in the Context of Neurological Disorders

Inherent Difficulties Related to the Study of the Nervous System For a successful application of SBD, a suitable animal model with objective and appropriate biomarkers must be established. When considering the molecular basis of a disorder, this may seem at first glance to be a fairly straightforward task as many molecular signaling cascades are conserved across the animal kingdom. For example, the components of the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway signaling pathway, which has been involved in a variety human diseases including brain cancer, neuronal injury following stroke, and depression in rheumatoid arthritis patients, can be found in the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and in the fish *Danio rerio*, as well as in mammals such as mice, rats and monkeys [78–80].

However, the structure and/or functional domains of proteins may also diverge during evolution with important consequences for disease biology. As an example, mutations in the human leucine-rich repeat kinase 2 (LRRK2) gene have been associated with an autosomal-dominant form of Parkinson's disease (reviewed in ref. [81]). Although *Drosophila* and *C. elegans* both express LRRK, their gene lacks an N-terminal repeat compared to the human LRRK2, which occurred later in evolution [82]. Studies the endogenous LRRK protein in these animal models, therefore, may not be appropriate to further our understanding of the role of LRRK2 in healthy humans and in those afflicted with Parkinson's disease. This is a good example of a protein that acquired additional functional domains during evolution, and these new functional domains may be important for the biological basis of a disease. It follows that the development of new therapeutics for the treatment of Parkinson's disease would reasonably focus on LRRK2 rather than LRRK if TBD were used. With SBD, the function of LRRK in the context of systems biology could correlate with the function of LRRK2. If true, and if a valid system approach could be conceived of, results from a lower organism could be used, perhaps in combination with TBD. Alternatively, it is possible that addition of new functional domains on a protein would affect its signaling and/or protein–protein interactions, in which case the pathway in which this specific protein is involved would need to be studied for SBD.

One example of an SBD strategy to streamline discovery for neurological disorders would be to pre-screen the initial chemical library to reduce its size and to begin the drug discovery process by using a hybrid approach that incorporates elements of TBD. Since 98% of compounds do not cross the BBB [52], performing an initial BBB screen on the entire chemical library would result in a focused library for neurological applications. Then, using knowledge of the molecular basis of a particular disorder, to the extent that it exists, some target-based screening might also be valuable. For example, positive modulation of a selected group of $GABA_A$ receptor subtypes by certain benzodiazepines and related therapeutics reduces anxiety in a safe and effective way. But all produce side effects such as drowsiness and sedation that limit their usefulness for day time anxiety. Whereas

the search for a selective anxiolytic free of sedative-hypnotic side effects has been driven by a TBD approach based upon $GABA_A$ receptor α2 subunit selectivity (reviewed in ref. [83]), ocinaplon was found to be a non-sedating anxiolytic in human subjects but surprisingly, relatively non-selective towards the α1, α2 and α3 subunit-containing $GABA_A$ receptors [84].

In the search for new anxiolytics, a hybrid approach in which BBB-permeable compounds are screened for activity against the benzodiazepine-responsive $GABA_A$ receptor subtypes could be effective. Instead of focusing on subtype selectivity as in TBD, compounds identified through HTS could be tested using the SBD approach. Following animal toxicity studies and phase I clinical trials, compounds that are likely to be effective in humans could be identified using system response profiling. It would seem that the search for new treatments for anxiety disorders and other neurological disorders would benefit greatly from future improvements in technologies for high-resolution analysis of nervous system function that are applicable to the intact human brain and spinal cord. Using these technologies nervous system responses in patients and normal controls could be generated. Drug candidates could then be evaluated in a small number of subjects using focused clinical trials to identify lead compounds that bring the patient's nervous system response profile toward that of a normal control. This approach would likely yield compounds acting on multiple targets but may improve future success rates.

Expanding the scale to a specific group of cells or an anatomical region containing a physiological pathway that is known to correlate with or be critical in the disease biology could be used. For example, the development of new therapeutic agents in the area of cognitive enhancement would fulfill an important unmet medical need. The hippocampus and parahippocampal region of the medial temporal lobe are well known to be important for declarative memory, the type of memory involved in remembering places, time, and events, as well as the emotions associated with these situations. One of the most famous examples for the association of the medial temporal lobe with declarative memory is provided by H.M., a patient with intractable epilepsy, who had his parietal temporal lobe surgically removed bilaterally in 1953 [85]. While H.M.'s seizures became manageable, he suffered severe anterograde amnesia, with partial retrograde amnesia, making him unable to complete episodic or semantic memory tasks, both of which are part of declarative memory (reviewed in ref. [86]). However, his short-term memory and ability to acquire new motor skills remained intact. Subsequent lesion studies in monkeys and rodents have confirmed the importance of the hippocampus and parahippocampal region for declarative memory, although the specific role of each individual anatomical region within the medial temporal lobe remains controversial (reviewed in ref. [87] and [88]).

Divergences in studying declarative memory across species may be explained in part by evolutionary divergences in the anatomical structures involved. Looking across mammalian species, the hippocampus appears to be well conserved both in its anatomical organization and in the intrinsic connections between its cornu ammonis (CA) field and dentate gyrus [89]. However, small variations can be observed in the parahippocampal region, and further disparities are found in the neocortex, which provides input to the hippocampus via the parahippocampal region. These differences may arise from connectivity patterns, cortical size and/or laminar stratification. Hence, if sensory information coming to the hippocampus from the neocortex differs across species, it is a strong possibility that the behavior of the animal, influenced by the hippocampal output, will also differ.

While some behaviors, such as recollection of previous events, may differ somewhat between species, other demeanors are strictly human and even more difficult to model in animals. An example of such behaviors found in schizophrenia, which includes positive (hallucinations, delusions, thought disorders), negative (anhedonia, lack of emotion and motivation, catatonia), and cognitive (attention and memory impairments) symptoms [90] that have yet to be reproduced in a comprehensive animal model. Models currently used can be classified as either predictive if they can be used to predict the therapeutic ability of a drug; or partially homologous if they can recapitulate some of the symptoms observed in humans [91]. One criticism associated with using predictive models is that they may miss drug candidates acting via a novel mechanism because these animal models are established and validated using currently available therapeutics. In addition, predictive models may be using behavioral assessment that represents only a minor problem in schizophrenic patients [91]. In the case of partially homologous models, in the absence of a better understanding of the disease biology of schizophrenia, it is difficult to assess whether treating the symptom(s) or objective responses represented in the animal model will truly yield an effective therapeutic in human patients.

Identification of new therapeutics for neurological disorders poses a problem in that there is often a lack of evolutionary conservation in the biological processes involved. While this can be solved at the molecular level by studying the human components involved in a pathway, it is difficult to establish a convincing parallel between information obtained from animal models in rodents and non-human primates and the results obtained in clinical trials. Furthermore, as shown in

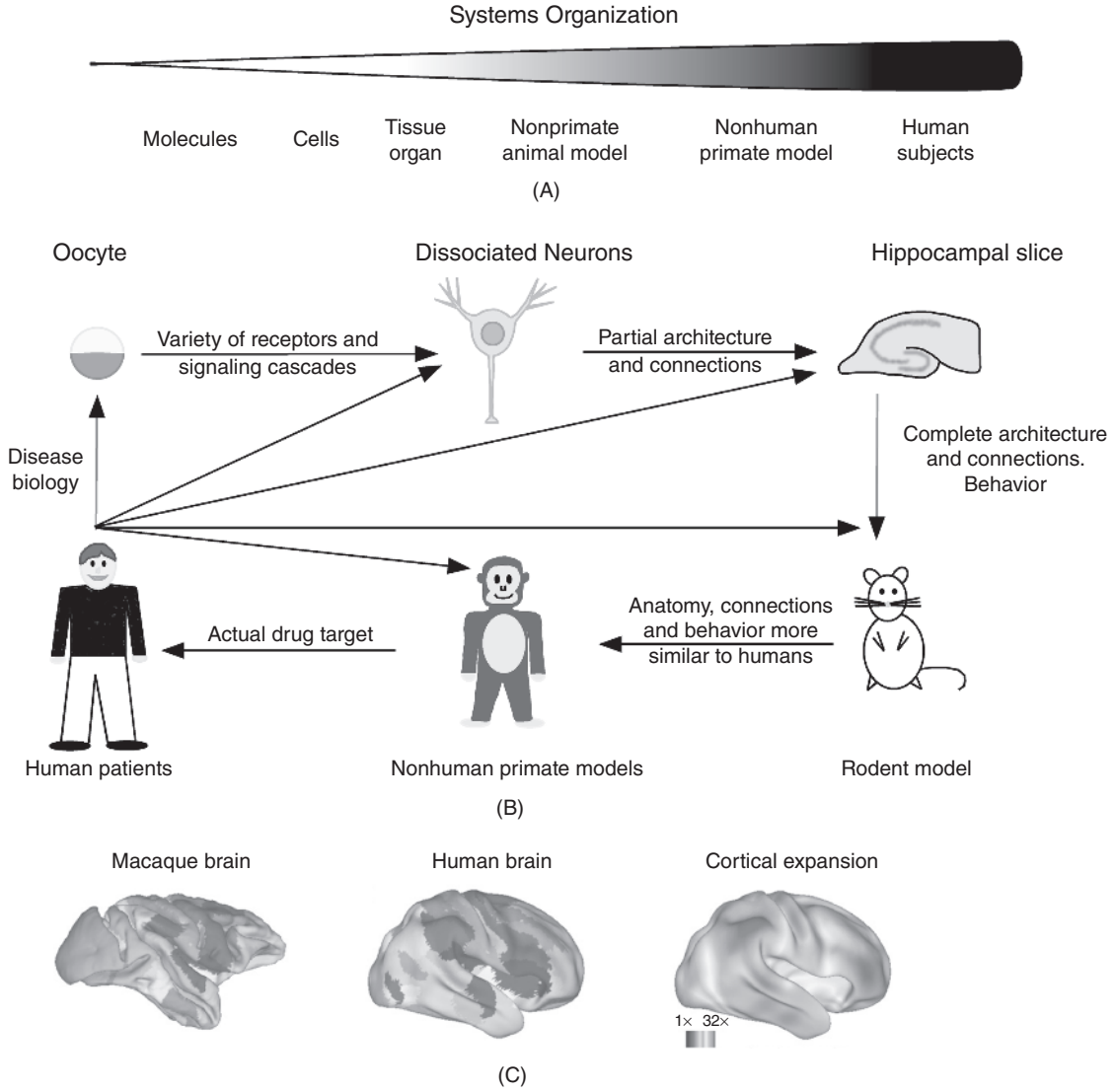

Figure 1.5 A systems pharmacology view of a platform for drug discovery in the nervous system. (A) The systems used for drug discovery can be arranged by increasing organization level. (B) Example of an adaptation of the organization level scale for drug discovery oriented towards neurological disorders. Although drug discovery programs may start at different steps of the process outlined here, a number of adaptations must be made when going from one model to the next. (C) There are a number of differences even between the human and macaque brain. PALS (population-average, Landmark, and Surface-based) rendering of the macaque and human brains are shown along with functional areas defined by Lewis and Van Essen [94]. Based on the localization and size of those functional areas, a map of cortical expansion can be designed. (Reproduced from ref. [92].)

Figure 1.5, discrepancies can arise at multiple levels, making it even more difficult to find an accurate animal model, even in species such as the macaque which is closely related to the human.

On the use of Integrative Databases as a Tool to Facilitate SBD for Neurological Disorders Since the use of animal models is essential to drug discovery and development, one SBD approach would be to track and record differences and similarities between subregions of the nervous system of different species. This includes their wiring diagram at strategic developmental stages depending upon the disorder to be treated. This neural systems-level information on circuitry could then be linked to anatomical information with functional data such as electrophysiology, behavior, functional magnetic resonance imaging (fMRI), gene expression and neurochemistry. Since there is a need to represent a

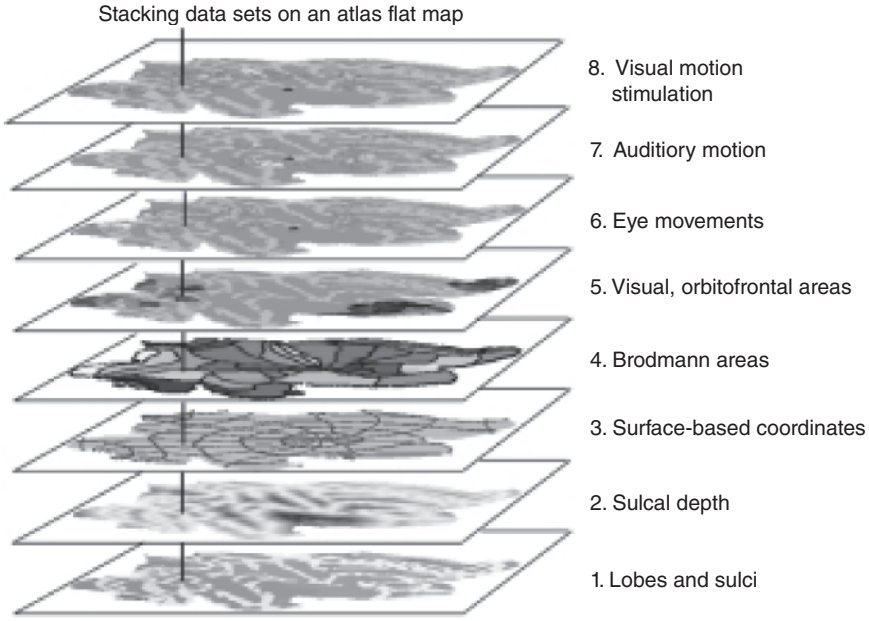

Figure 1.6 Example of a brain atlas. Anatomical and functional information may be displayed on a flat map (reproduced from ref. [97].)

large amount of annotated anatomical information, databases referred to as multimodal brain atlases have emerged (Fig 1.6) [92]. Electronic brain atlases offer several advantages over print atlases in that they provide a dynamic link between structure and function, which is easily accessible, searchable, updated and linked to other databases and resources [93]. In addition, whereas traditional atlases were based on post-mortem studies of one or a few brains, multimodal atlases incorporate information from MRI and allow the creation of population averages and probabilistic analysis.

There are a number of databases currently available which may help in the application of SBD to neurological disorders. Human and macaque surface-based brain atlases using flat images and/or 3-dimentional reconstructions (reviewed in ref. [93]) are derived mainly from imaging studies, while other databases include different types of information. The Allen Brain Atlas provides a comprehensive analysis for the expression of 20,000 genes in the brain of adult (post-natal day 56, P56) male mice [95]. Searches in this freely available online atlas provide images from in-situ hybridization, annotated for major brain structures, and links for the gene or protein of interest in other databases. The GenePaint Atlas also provides information on gene expression in the mouse brain but includes brain from different developmental stages [96]. Although the number of genes covered is smaller than that of the Allen Brain Atlas, information on spatio-temporal expression of genes can be especially important for developmental disorders such as autism and schizophrenia. In addition, the database is updated weekly, and researchers can submit requests for candidate genes [98].

Another issue frequently encountered when studying a developmentally regulated process is the "conversion factor" between the brain of two species. To address this question, Clancy and colleagues used neuroinformatics to translate brain development data across 10 species including mice, rats, cats, macaques and humans [99]. Since different brain regions mature at different times in different species, this program further allows to compare two animals for cortical events, limbic events, and non-cortical/limbic events. In the context of SBD, this would help establishing a valid animal model for developmentally regulated processes and diseases.

1.4 CONCLUDING REMARKS

The science of pharmacology has progressed tremendously since its inception. Microbial infections that were once fatal can now be treated with antibiotics and other conditions for which there is still no cure, such as type I diabetes and certain breast cancers can be managed in many patients using a number of approaches including the appropriate medication. However the promise of a horn of plenty filled with new biology "wonder drugs" based on modern discoveries of pharmacology com-

bined with advances such as the sequencing of the human genome remains at the end of the rainbow. One could even wonder whether the outlook for the development of breakthrough therapeutics does not look grim with the continued decrease in yearly new drug approvals over the past decade combned with increased legal, social and political pressure. To resolve this issue, the pharmaceutical industry may need to bring a shift in its drug discovery and development strategies, whether it is for the generation of new small molecules, biotherapeutics, or nucleic-acid-based therapeutics and/or more productive focused clinical trials. Although TBD has seemed, in theory, the way to increase efficacy while reducing side effects of new therapeutics, a look at the current rates of success and failure for new drug candidates, as well as their associated costs, seems to suggest otherwise. An alternative approach, as suggested here, would be to consider enhancing SBD strategies within the drug discovery scheme. This may reduce the fraction of drug candidates that have inadequate efficacy and/or induce significant side effects, decreasing the average cost of discovery and development that accrues as drugs emerging from company pipelines fail pre- and postmaketing. At the same time it is possible to generate compounds that act through novel mechanisms or via combined actions at several targets. Similarly, a polypharmaceutical approach in which a novel combination of approved therapeutics may be identified by SBD provides practical advantages if the screening process could be shown to yield substantially higher value lead therapeutic combinations. However, the application of SBD may be particularly challenging in certain diseases where it is difficult to accurately represent the spectrum of symptoms observed in humans within an animal model. To remedy this, an important objective for R&D is to identify better biomarkers for disease progression, develop a better understanding of cross-species differences and, based on this information, to develop more valid animal models that can be monitored using the same biomarkers carried through to clinical trials.

Nowhere is the disconnect between human disease and animal models for disease more apparent than for those disorders based upon dysfunction of the nervous system. As just one example, the nervous system challenges biomedical science to break the barrier of disconnect between the anxiety perceived by the patient with generalized anxiety disorder and the animal models for it, using biomarkers in order to guide discovery. Regardless of the approach taken, efforts must be made to diversify the treatment options available and to provide new therapeutics for conditions where there is a medical need. For many neurological disorders, such as schizophrenia and depression, treatment options are associated with severe side effects and are effective in only a fraction of the population, whereas in other conditions, such as Alzheimer's and Parkinson's diseases, therapeutics only reduce somewhat the appearance of symptoms in some patients. Whereas therapeutics exist for the treatment of anxiety disorders, convulsive disorders and sleep disorders, the existing treatments are palliative and burdened with untoward side effects. In addition, there is no treatment available for a number of neurological diseases including spinal muscular atrophy, a hereditary motor neuron disorder. While the nervous system is complex and presents with additional challenges, such as the BBB and the BNFB, which are absent in other organs, it is critical that efforts be invested in this area to overcome this lag in the development of new therapeutic agents. It is our hope that advances in SBD, combined with biomarker development, validated animal models for diseases, and enhancements in the design, surveillance and interpretation of clinical trials will lead to the discovery of therapeutic cures rather than palliative treatments for disorders and diseases that afflict us.

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