Editorial

Dear Colleagues,

Clinical neuroscience is a field in which subjective elements of human psychology and objective data about the brain exist alongside each other. The range of this scientific discipline has become extensive, and thus specifically targeted paraclinical investigations have emerged, with the opportunity to validate clinical and nonclinical observations.

Two major questions now arise:

- First, the understanding of the pathophysiology of central nervous system diseases and the validation of quantifiable and reproducible indicators relevant to their identification.
- Second, the identification, as early as possible during the development of a drug, of its molecular targets and the consequences of its action on these targets, as well as its pharmacodynamics.

Currently, there is no commonly agreed-on exhaustive research approach which can provide definite answers to these questions, but certain approaches, highlighting the mechanism of diseases, and the mode of action of products designed to treat them, have increased our knowledge in the area. These approaches, based on both clinical and fundamental technologies, (neurobiology, pharmacodynamics, in vivo and in vitro models, dynamic and functional cerebral imaging, etc) lead to what is known as "proof of concept." Proof of concept is achieved when a series of techniques provide early confirmation of the validity of a given hypothesis concerning a disease or its treatment.

There are three principal fields for the application of proof of concept:

- Preclinical (animal studies)
- Clinical studies in healthy volunteers
- Clinical studies in patients.

The characteristics of the mechanisms of mental diseases, as well as those of new medications, must be appropriately taken into account to provide us with relevant answers to the many questions arising during drug development. Moreover, this development should involve the smallest number of subjects, with the least invasive investigations possible, and the minimum doses of the product.

We have selected, for this issue of *Dialogues in Clinical Neuroscience*, several original approaches describing new facets of this area; these contributions are from a selection

Editorial

of remarkable authors, whom I would like to thank warmly for their contribution. I would also like to thank Pierre Schulz for being the Coordinating Editor of this issue.

This also represents for me an opportunity to point out that Professor Manfred Ackenheil shared with me, over many years, ideas and projects on the theme of proof of concept. Manfred Ackenheil has just passed away; it is with great sadness that I bring you this news, both on my own behalf and that of the Editorial Board of *Dialogues in Clinical Neuroscience*, of which he was a founding member. He was working as the Coordinating Editor of issue no 32 of our journal, and this issue, to be published in early 2007, will be an "In Memoriam" issue dedicated to Manfred Ackenheil—a tribute to a man of science and to a friend.

Jean-Paul Macher, MD

Dialogues in Clinical Neuroscience is a quarterly publication that aims to serve as an interface between clinical neuropsychiatry and the neurosciences by providing state-of-the-art information and original insights into relevant clinical, biological, and therapeutic aspects. Each issue addresses a specific topic, and also publishes free contributions in the field of neuroscience as well as other non–topic-related material. All contributions are reviewed by members of the Editorial Board and submitted to expert consultants for peer review.

Indexed in MEDLINE, Index Medicus, EMBASE, Scopus, Elsevier BIOBASE, and PASCAL/INIST-CNRS.

EDITORIAL OFFICES

Editor in Chief

Jean-Paul MACHER, MD

FORENAP - Institute for Research in Neuroscience and Neuropsychiatry
BP29 - 68250 Rouffach - France
Tel: + 33 3 89 78 70 18 / Fax: +33 3 89 78 51 24

Secretariat, subscriptions, and submission of manuscripts

Marc-Antoine CROCQ, MD

FORENAP - Institute for Research in Neuroscience and Neuropsychiatry
BP29 - 68250 Rouffach - France
Tel: +33 3 89 78 71 20 (direct) or +33 3 89 78 70 18 (secretariat)
Fax: +33 3 89 78 51 24 / E-mail: ma.crocq@ch-rouffach.fr

Annual subscription rates: Europe €150; Rest of World €170.

Production Editor

Catriona DONAGH, BAppSc

Servier International - Medical Publishing Division
192 avenue Charles-de-Gaulle
92578 Neuilly-sur-Seine Cedex - France
Tel: +33 1 55 72 32 79 / Fax: +33 1 55 72 62 57
E-mail: catriona.donagh@fr.netgrs.com

PUBLISHER

Les Laboratoires Servier
22 rue Garnier - 92578 Neuilly-sur-Seine Cedex - France
E-mail: mail.dialneuro@fr.netgrs.com

ISSN 1294-8322

Printed on acid-free paper.

Design: Christophe Caretti / Layout: Graphie 66
Imprimé en France par SIP
1, rue Saint Simon - 95310 Saint-Ouen-l'Aumône

Contents

ISSUE COORDINATED BY: *Pierre Schulz*

Contributors

Ralf Herwig, PhD

Author affiliations: Max Planck Institute for Molecular Genetics, Department of Vertebrate Genomics, Berlin, Germany

Eberhard Fuchs, PhD

Author affiliations: Clinical Neurobiology Laboratory, German Primate Center, Göttingen, Germany; Department of Neurology, Medical School, University of Göttingen, Germany

Michael Spedding, PhD

Author affiliations: Deputy Research Director, Chairman NC-IUPHAR, Experimental Sciences, Servier Research Institute, Suresnes, France

Thomas A. Ban, MD, FRCP(C)

Author affiliations: Emeritus Professor of Psychiatry, Vanderbilt University, Nashville, Tenn, USA

Bryan L. Roth, MD, PhD

Author affiliations: Departments of Biochemistry, Psychiatry, Neurosciences, and Oncology, National Institute of Mental Health Psychoactive Drug Screening Program, Case Western Reserve University Medical School, Cleveland, Ohio, USA (Current affiliation: Departments of Pharmacology, Medicinal Chemistry and Natural Products, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA)

Luc Staner, MD

Author affiliations: Centre Hospitalier, Secteur VIII, Rouffach, France

François Girardin, MD

Author affiliations: Unit of Clinical Psychopharmacology, Geneva University Hospitals, Chênes-Bourg, Geneva, Switzerland

Barbara K. Lipska, PhD

Author affiliations: Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Md, USA

In this issue...

Brain biochemistry is rich and complex, and brain functions are also varied, complex, and interdependent. Drugs for treating central nervous system (CNS) diseases act on the biochemical machinery of the brain, on cell membrane or nucleus receptors, on second messengers, and on neurotransmitters. This leads to changes in macroscopically measurable brain functions such as perception, motor activity, memory, emotion, consciousness, and many other functions that enable our adaptation to our changing internal, physical, and social environments.

The first CNS drug discoveries during the 1950s and 1960s were serendipitous to some extent, but also guided by astute hypotheses. At that time, a series of useful drugs could be developed by one researcher, or by a rather small team, which has become near to impossible now. Several drugs that were discovered in those times remain standards, to be used as comparatives in the current evaluation of the clinical efficacy of drugs in development.

Researchers in pharmaceutical sciences and industries now have a huge knowledge base in fundamental and clinical neurosciences, from which to develop innovative drug treatments for neurological or psychiatric diseases and disorders. Indeed, nowadays, a pharmacological compound is described in an exhaustive manner, covering all its influences (by analogy with an antibiogram): ie, a receptorgram, a transportergram, an enzymogram, a genogram, a proteinogram, a metabogram; these terms concern the list of all biochemical constituents that are changed during drug administration. To this biochemical level of description, one can now add a physiological level, ie, the drugs' influences on neuronal networks devoted to given CNS functions. Neuroimaging technologies are relevant tools at this level of analysis. As an illustration of a physiological description of pharmacodynamics, some antidepressants influence the automatic and nonconscious perception of others' emotions, by dampering the activity of the amygdala and related structures. The final goal of neurologic or psychiatric drug treatment is the control of symptoms, acutely, chronically, or with a preventive goal, ie, to act in a bottom-up manner, from molecules to mind.

In this issue of *Dialogues in Clinical Neuroscience*, the authors describe how researchers look for new therapeutic compounds, ie, how they explore unknown domains to discover those structure/activity relations that could provide therapeutic effects with minimal side effects. An overall conclusion to be drawn from these articles is that the challenge of new drug discovery is immense, and requires intelligence and the use of shortcuts, as well as a thorough and cautious approach. To these research competences, one should also add real administrative talent. A group of lazy men once returned after a terrible day's work: there was work for at least six people, but fortunately there were twelve of them! Looking at the contributions by the authors of this issue, I am persuaded that the reverse situation prevails in the domain of drug discovery: a small number of people have to achieve a lot.

A ***State of the art*** paper on the expression profiling of drug response, from genes to pathways, by Ralf Herwig and Hans Lehrach, provides a relevant introduction to the theme of drug discovery and proof of concept. Indeed, the domain of therapeutics will benefit from innovative ideas on how to identify targets of medication. The recent functional genomic technology with DNA arrays or microarrays, ie, the capacity to measure the transcription of thousands of genes into RNA molecules, enables us to study the configuration of genomic changes induced by a given drug treatment. This advance offers a realistic approach to pharmacology, in the sense that the pharmacodynamics of drugs is now addressed at its actual level of complexity, taking into account, at least theoretically, all changes in protein synthesis. The authors describe the DNA transcription techniques and then review the difficulties and the pitfalls of these techniques at several levels, ie, in the laboratory, in data analysis, in statistics, in standardization, and in the necessary international collaboration between institutions. The authors then give their view as to how many new drugs could be found on the basis of well-defined molecular targets; they also mention avenues for the integration of functional genomics techniques with other techniques, with the aim of understanding the successive steps from DNA transduction to neuronal metabolism, to neuronal networks and then to global physiological systems, analyzed macroscopically. Functional genomic technology can lead, not only to new medications in neurology and psychiatry, but also to a more efficient prediction of the efficacy and safety of a given medication for a given patient.

In his ***Pharmacological aspects*** paper, Michael Spedding discusses new directions for drug discovery. First, he

reminds us that the development of a compound that can be registered for the treatment of human diseases is long, difficult, and expensive. Efficacy and safety requirements are obvious and inescapable aspects of this development, explaining in great part why few of the compounds initially screened ever reach the market. The author presents a series of techniques for the screening of compounds, some of them quite recent, others known for decades; these techniques test different domains of inter- and intracellular transfer of biological information (membrane or nuclear receptors, ion channels, messengers and other signaling molecules, enzymes, etc) that permit the identification of potential new compounds, called lead compounds, and the choice of the most promising of these compounds, ie, lead optimization. This optimization should rely on specific nodes, ie, on relevant aspects of molecular cascades, changes in gene expression, changes in neurotransmitters, and changes in brain physiology. The effort is wide-ranging, and Spedding explains the advantages of partnerships between researchers from university and industry, towards the goal of rapid and efficient drug discovery processes.

Molecular biology techniques are applied in all fields of medicine. Bryan L. Roth writes about their place in drug discovery, in a ***Pharmacological aspects*** paper on antipsychotic drug discovery; he asks whether promises have been fulfilled or not. Many drugs still prescribed to patients suffering from psychiatric disorders were developed at a time when no molecular techniques were included in the screening and development phases of new drugs. The promises of molecular biology compounds in the domain of schizophrenia. How does the mode of action of marketed antipsychotics and of potential new compounds fit into our understanding of the molecular changes underlying or accompanying schizophrenic disorders? Which pharmacological mode of action would be critical, in the sense of being more or less directly bound to the mechanism of schizophrenia, acting at a "critical node" of this disorder? Should drug therapy of schizophrenia aim at one or at several biochemical targets? Roth suggests that complex disorders such as schizophrenia, that have complex genetics, might also benefit from complex pharmacology, ie, from drugs acting on the D_2 dopamine receptor, plus on many other targets. He also suggests that drug development based on recent discoveries about the genetics of schizophrenia might open up a new area of psychopharmacology.

In another ***Pharmacological aspects*** paper, François Girardin explains how unbound (or free) drug concentration in the brain might differ from that in other organs, because of the existence of membrane transporter proteins, located in cell membranes of the blood brain barrier. These proteins, for example P-glycoprotein, were discovered a few years ago in the brain and in other organs with barriers or serving as barriers, such as the digestive tract, renal tubules, or circulating blood cells. Among the consequences of this discovery, one can include better understanding of individual differences in drug disposition due to polymorphism of the transporters, of drug/drug interactions leading to enhanced or decreased drug concentration in the brain, of disease/drug interactions having the same consequences. Several of these consequences are of a clinically relevant magnitude. Membrane transporter proteins are now a piece of the puzzle of drug development for CNS diseases.

Animal models of diseases have an important role in the exploration of the mechanisms of mental disorders or their treatment. Eberhard Fuchs and Gabriele Flügge address this role in their ***Pharmacological aspects*** paper on animal models for the simulation of depression and anxiety. Such models help explore whether depression results from mild chronic stress, from loss of hierarchical position in the group, from inadequate hedonic tone, or from loss in significant affiliation with other members of the group; many of the above causal factors could induce changes in given and specialized neuronal networks and neuroanatomical systems. This paper offers a clear summary of models of depression and anxiety, with the information organized according to the characteristics of the models themselves, to their validity using different criteria of evaluation (such as the relevance of the model to normal behaviors, to the physiological changes accompanying the test conditions and the human diseases, to the influence of medications). The authors suggest choosing and using a number of animal models, rather than just a single preferred one. Clinicians with little interest in basic research should also appreciate this review, since so many animal models in psychiatry are based on conditions that are homologous to our human life and social organization; philosophically, animal models of depression or anxiety serve as a reminder of the mammalian structure of human behaviors and emotional responses. Scientifically, these models are among the techniques aiming at the elucidation of the mechanisms of mood and anxiety disor-

In this issue...

ders and at the discovery of drugs to alleviate these extremely painful experiences.

"Serendipity" is a wonderful word, and an interesting concept pertaining to the role of chance, or hazard, in human discoveries. Thomas A. Ban, in a ***Clinical research*** paper, reviews the history of early drug discovery and the role that serendipity has had in these achievements. He illustrates how, on one hand, mere hazard can participate in major discoveries and how, on the other hand, some discoveries result from a succession of rational decisions based on astute observations. Quoting Goethe, the author concludes that discovery needs luck, invention, and intellect. These components are probably mixed together in modern research, just as they have been in the past.

An important ***Clinical research*** paper is that by Luc Staner, on the question of surrogate outcomes in psychopharmacology. Surrogate outcomes, or markers, concern the question of how to predict the effects of a treatment, its benefits, and its side effects, before large-scale clinical trials are carried out. This is of great importance for all of medicine, and there exist several surrogate markers in the fields of oncology or cardiovascular medicine. In the field of neurology there are few surrogate markers, and none in the field of psychiatry, although many endophenotypes have now been described, for example pertaining to the abnormal way in which the brain of a person with schizophrenia handles information pertaining to perceptions. The scientific and regulatory frameworks for the use of surrogate markers in psychiatry should not differ from those in other domains of medicine. Finding a surrogate marker that can be used as a primary variable in clinical trials of psychotropic medication is a worthwhile challenge in clinical psychopharmacology.

Pierre Schulz, MD

Expression profiling of drug response—from genes to pathways

Ralf Herwig, PhD; Hans Lehrach, PhD

Recent reports have highlighted the imbalance between rising costs in drug discovery and the production of new molecular entities for the market,[1,2] leading to a long-term loss of efficiency. Remarkably, this decline in productivity has occurred despite the fact that biomedical research benefits from large governmental and private investments, and despite the comprehensive improvements in our knowledge of human genes resulting from large sequencing projects.

The tremendous efforts that have to be invested for drug target identification, follow-up validation studies, and clinical trials, in combination with the high failure rate as a consequence of individual response to drugs, has imposed high costs on the development of drugs. Understanding individual response to a drug, what determines its efficacy and tolerability in the patient's body, is the major bottleneck in drug development and

Understanding individual response to a drug—what determines its efficacy and tolerability—is the major bottleneck in current drug development and clinical trials. Intracellular response and metabolism, for example through cytochrome P-450 enzymes, may either enhance or decrease the effect of different drugs, dependent on the genetic variant. Microarrays offer the potential to screen the genetic composition of the individual patient. However, experiments are "noisy" and must be accompanied by solid and robust data analysis. Furthermore, recent research aims at the combination of high-throughput data with methods of mathematical modeling, enabling problem-oriented assistance in the drug discovery process. This article will discuss state-of-the-art DNA array technology platforms and the basic elements of data analysis and bioinformatics research in drug discovery. Enhancing single-gene analysis, we will present a new method for interpreting gene expression changes in the context of entire pathways. Furthermore, we will introduce the concept of systems biology as a new paradigm for drug development and highlight our recent research—the development of a modeling and simulation platform for biomedical applications. We discuss the potentials of systems biology for modeling the drug response of the individual patient.

Dialogues Clin Neurosci. 2006;8:283-293.

Keywords: *drug discovery; functional genomics; microarray; bioinformatics; data integration; database; systems biology*

Author affiliations: Max Planck Institute for Molecular Genetics, Department of Vertebrate Genomics, Berlin, Germany

Address for correspondence: Dr Ralf Herwig, Max Planck Institute for Molecular Genetics, Department of Vertebrate Genomics, Ihnestr. 73, D-14195 Berlin, Germany
(e-mail: herwig@molgen.mpg.de)

Selected abbreviations and acronyms

AD	*Alzheimer's disease*
ALS	*amyotrophic lateral sclerosis*
DRPLA	*dentatorubral-pallidoluysian atrophy*
GEO	*gene expression omnibus*
GO	*gene ontology*
GPCR	*G-protein-coupled receptor*
HD	*Huntington's disease*
PCR	*polymerase chain reaction*
PD	*Parkinson's disease*
SAGE	*serial analysis of gene expression*
SOP	*standard operating procedure*

clinical trials. When a drug is delivered through the body, each individual reacts differently in terms of intracellular response and metabolism. A prominent example is seen with the cytochrome P-450 enzymes, a family of drug-metabolizing enzymes that may either enhance or decrease the effect of different drugs, dependent on the genetic variant.[3] Thus, the individual genetic composition of the patient has become a major issue in studying drug targets and responses to medical treatment.

Microarrays are the state-of-the-art platform for screening the genetic composition of the individual patient. This technology offers the chance to acquire the complete state of gene expression[4-6] and to identify genes and pathways that are affected by the treatment.[7,8] On the other hand, high-throughput technologies such as microarrays are also a part of the problem. The new technologies have led to an increasing amount of heterogeneous (and often conflicting) data, corresponding to an increasing amount of potential drug targets.

Microarray experiments are "noisy" by nature, and must be accompanied by solid and robust data analysis components. This task has been part of bioinformatics research since the advent of this new discipline. The components of microarray analysis range from low-level analysis, explorative statistics to higher-level analysis involving additional data, annotation, and knowledge in order to embed the gene expression data in a functional context. The main purpose of data analysis is to filter the information and to enrich the level of information complexity from single gene markers to biological pathways.

This article will discuss the state-of-the-art deoxyribonucleic acid (DNA) array technology platforms and the basic elements of data analysis and bioinformatics research in drug discovery, developed by us and others. Apart from the single-gene analysis we will present a new method for interpreting gene expression changes in the context of the pathways involved. Recent microarray applications for neuroscience will be considered, and the particular challenges for gene expression analysis of the brain will be discussed. Furthermore, we will introduce the concept of systems biology as a new paradigm for drug development and highlight our recent research—the development of a modeling and simulation platform for biomedical applications. This research field, which shows great potential for modeling the drug response of the individual patient, will deliver valuable hypotheses for personalized drug treatment and therapy monitoring in the medium to long term.

DNA array platforms for gene expression profiling

DNA arrays are the most common gene expression profiling technology. A DNA array consists of a solid support (nylon membrane, glass chip) that carries DNA sequences representing genes—the probes. In hybridization experiments with the target sample of labeled complementary ribonucleic acids (cRNAs) and through subsequent data capture a numerical value, the signal intensity, is assigned to each probe. Labeling is done either radioactively (phosphorus, ^{33}P) and detected with a phosphor imager or fluorescently (Cy3/Cy5 dyes) and detected with specific scanners. Chips are typically small (<2 cm^2) and allow the immobilization of tens of thousands of different gene representatives.

The most prominent DNA array technology is the Affymetrix GeneChip system.[9] Here, genes are represented by probe sets of short oligonucleotides (typically 11 to 20 25mers) that are distributed across their sequences. These oligonucleotides are synthesized in a highly specific manner at defined locations using a photolithographic procedure. After hybridization, the measured intensity for the represented gene is summarized across the different probes in the probe set. Affymetrix chips have emerged as the pharmaceutical standard, and are widely in use because of the highly standardized chip generation process. Whole-genome chips are available for a large number of organisms, such as human, mouse, rat, bovine, pig, etc. An experiment with Affymetrix technology is typically a single-channel experiment, ie, only one target sample is analyzed in one experiment.

An alternative technology is the Agilent system.[10] This relies on the immobilization of longer oligonucleotides (60mers) synthesized in situ at or near the surface of the slide by inkjet printing using phosphoramidite chemistry. These probes are highly specific for the represented gene and show, generally, better hybridization properties than systems with shorter oligonculeotides. Experiments are typically double-channel experiments, ie, two target samples are analyzed simultaneously, each labeled with a different cyanine dye and quantified with a separate scanning procedure.

A recent technological development is the Illumina BeadChip system[11,12] that utilizes an "array of arrays" format. Each array on the support contains thousands of wells into which up to hundreds of thousands of beads self-assemble in a random fashion. Specific 50-mer gene sequences concatenated with an address sequence recognize the beads and attach to them. After bead assembly, a hybridization-based procedure is used to map the array, to determine which bead type resides in each well of the array and to validate the performance of each bead type. An advantage of this technology is that several samples can be analyzed on the same chip, thus preventing experimental artifacts across chips or dye labeling procedures. For example, the recent HumanRef-8 chip offers the possibility of screening eight different samples in parallel.

Other commercial chip providers are Amersham Biosciences, NimbleGen, Febit, and Applied Biosystems. There are advantages and disadvantages of the above-mentioned platforms regarding hybridization specificity, sample target material needed, and other factors, as pointed out in a recent review.[13]

Historically, the first array technology was based on spotted cDNAs.[14-16] This technology is still extensively in use in the academic sector, but also in pharmaceutical research

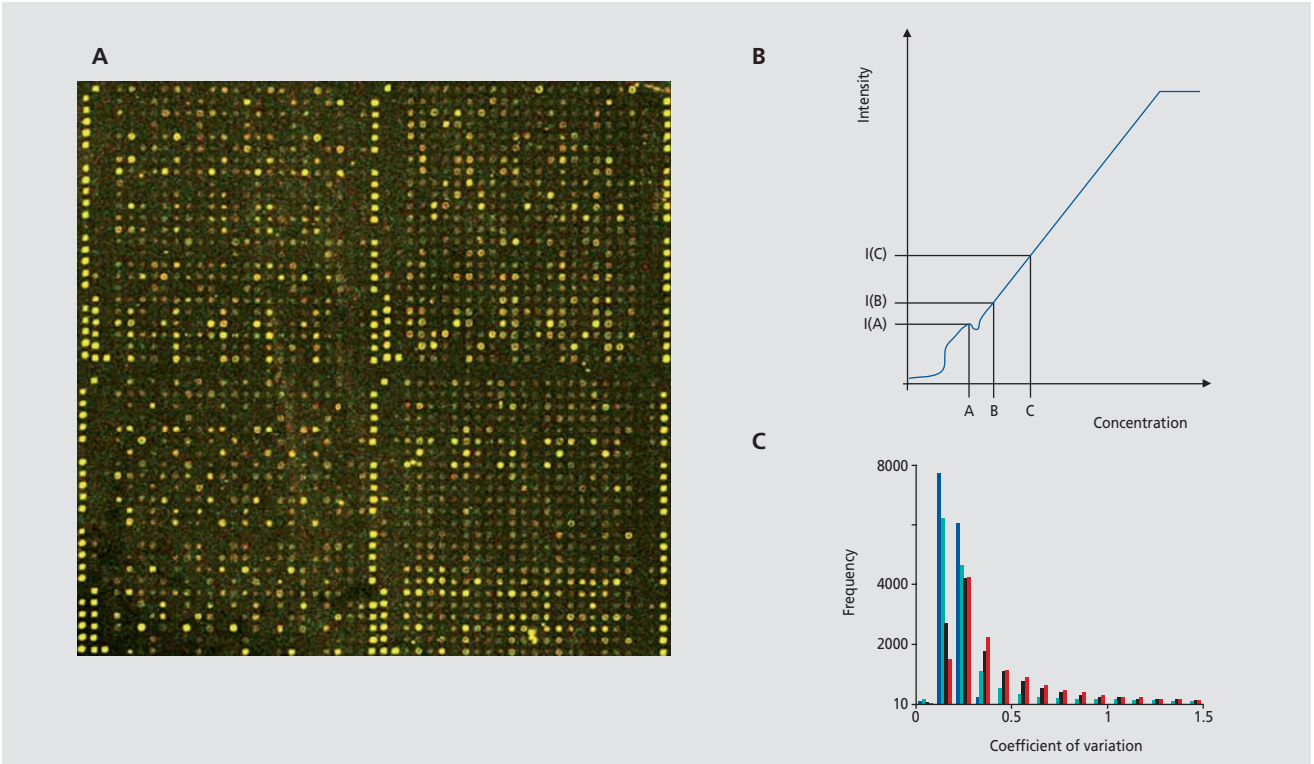


Figure 1. **A**: False-color image generated from a two-color hybridization on a cDNA array.[17] **B**: Linearity between concentration and measured signal intensity is the underlying assumption of microarray data analysis. Whereas the expression ratio of genes B and C yield a valid measure of the concentration differences, the ratio of genes A and B is misleading because of nonlinear deviations in the low intensity region. **C**: Histogram of the coefficient of variation for genes from simulated array images[26] using three different image analysis programs for data analysis that can be classified as manual (red), semi-automatic (black) and fully automatic (green). The blue bars show the counts for the simulated input data.

that involves probe sets not covered by standard array formats. cDNAs have a high variability in length (600 to 1500 bp) and are amplified using a polymerase chain reaction (PCR). PCR products are then transferred to the surface via contact printing by robotic devices *(Figure 1a)*.

The implicit assumption of all microarray studies is that the signal intensity measured with a specific probe is proportional to the number of molecules of the respective gene in the target sample. Changes in signal intensities are interpreted as concentration changes. It should be pointed out that the signal intensities are only crude estimators for the actual concentrations, and the interpretation as concentration changes is only valid if the intensity-concentration correspondence is approximately linear. Microarray measurements often show deviations from this assumption: for example, saturation effects; the spot signals are above a limit that no longer allows the detection of concentration changes or other nonlinearities if the concentration of the gene is below the detection limit of a microarray *(Figure 1b)*.

Whole-genome chips carry probes for (more or less) the entire genome. These chips are used typically in the beginning of a study when it is not clear what genes are responsible for the drug response of certain groups of patients (for example drug-sensitive and -resistant). For diagnostic purposes specific theme (or custom) chips are used that carry only a few marker genes. The use of custom microarrays for neuroscience applications has been discussed recently.[18]

There have been several studies comparing the performance of microarray platforms.[19-22] Most of these studies reveal a poor correlation in the global expression of the genes. This might be due to several reasons, such as hybridization sensitivity due to the different probe lengths, different chemical treatments, and different statistical methods in the readout of the scanned images. A further issue is the source of the probe sequences. Annotation and probe design typically differ with the background sequence database used by the provider. Currently, several competing collections of transcript sequences are available, and serve as the basis for probe annotation such as Unigene, Refseq, LocusLink, ENSEMBL, etc. Furthermore, probe design of the chip provider must be updated regularly. A recent study showed the potential misinterpretation of experiments performed with Affymetrix probe set assignments that are not updated to the latest genome annotations, and reported a 30% to 50% discrepancy in final lists of differentially expressed genes in several gene expression studies.[23]

Inherent in most technology platforms is software to read the digital image after the scanning process and to compute for each gene representative the intensity value.[24,25] Image analysis methods can be grouped into three different classes: manual, semiautomated, and automated methods. Simulation studies on systematically perturbed artificial images have shown that the data reproducibility increases with the grade of automation of the software *(Figure 1c)*.[26] However, for "noisy" images that show a very irregular structure, manual methods might be the best choice.

Data analysis components

Analysis of expression data comprises several modules that address different questions relevant for drug response screening.[27] The most important tasks are:

- to identify genes that are differentially expressed when comparing two or more conditions (for example, groups of patients resistant or sensitive to a certain drug)
- to identify common gene expression patterns that classify individuals accordingly
- to identify relevant pathways explaining the expression patterns.

Regarding the complexity of the resulting information, the major goal of data analysis is filtering the many thousands of uninformative genes to a set of informative markers, networks, and pathways that are relevant for the problem under analysis *(Figure 2a)*.

Data from microarray experiments typically come out in the form of a table with raw data, ie, the measured intensity values. This raw data is not easily comparable across experimental replicates, so that some *data preprocessing* (or normalization) is necessary. The task of normalization is the elimination of influencing factors that are not due to the probe-target interaction, such as labeling effects (different dyes), background correction, pin effects (spotting characteristics), outlier detection (cross-hybridization of oligonucleotide-probes), etc, thus making signal values comparable across different experiments *(Figure 2b)*. Different algorithms and methods have been proposed to fulfill these tasks.[28-34]

The *identification of differentially expressed genes* between two or more experimental conditions is typically based on two-sample location tests. This setup utilizes replicated experiments with independent samples. The power of such tests is heavily dependent on the number of experimental replicates *(Figure 2c)*. These tests can be used to assign to

each single gene a *P* value that judges the significance of the fold change. Here, it is notable that this *P* value is only valid if the distributional assumptions are valid. For example, if a Student's *t*-test results in a significant *P* value, the implication that the corresponding gene is differentially expressed is only true if both sample series are Gaussian-distributed and have equal variances. Usually, these assumptions do not hold in practice but, strikingly, in most studies this fact is entirely ignored. In our studies we rely therefore on nonparametric alternatives[17,35] *(Figure 2d)*: Wilcoxon's rank sum test is based on the ranks of the replicates rather than on the actual signal values. This test (and other tests based on linear rank statistics such as the van der Waerden test) is preferable to the parametric t-tests if the distributional assumptions cannot be proven to be Gaussian. Furthermore, for "noisy" data this test yields more robust results since it is less sensitive against outlier values. For larger sample sizes, ie, >25 replicates, we can approximate the *P* value of the Wilcoxon rank test by the standard normal distribution. However, most practical applications will be based on a rather smaller number of observations (sample sizes in the order of 4 to 12). Therefore, those *P* values must be calculated exactly. This can be done using a recursive method.[36]

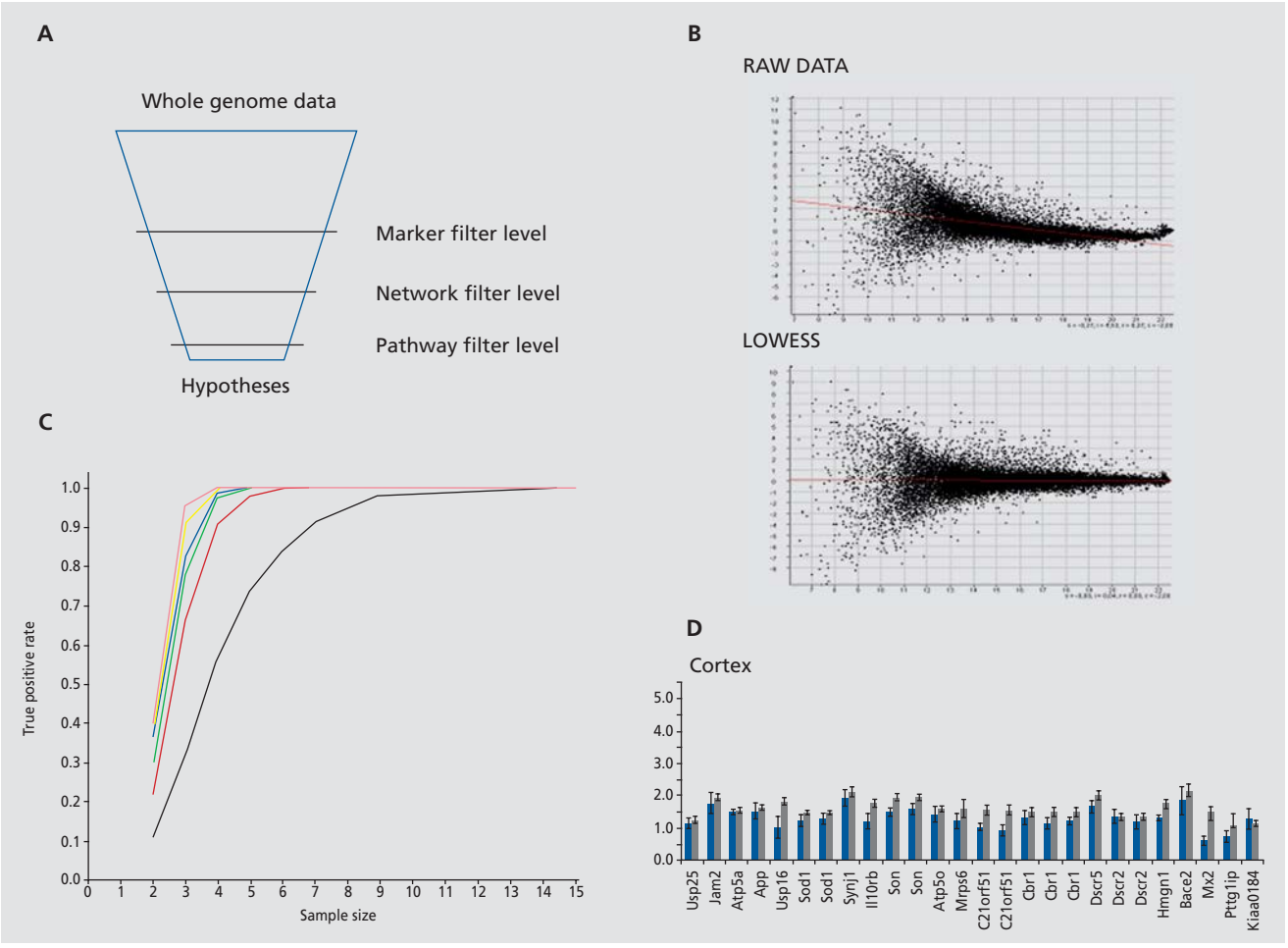


Figure 2. **A**: Schematic description of the biomarker discovery process. **B**: Nonlinear dependencies of fold change (Y-axis) and signal strength (X-axis) in raw data and LOWESS normalization for the compensation of these effects. This method fits the data sets by local polynomials using weighted least squares. **C**: Dependency of detection power for expression differences (Y-axis) from the number of experimental replicates. Different curves correspond to different expression ratios: 1.5 (black), 2 (red), 2.5 (green), 3 (blue), 5 (yellow) and 10 (magenta). **D**: Robust statistical testing identifies even small expression changes (~1.5). Microarray expression changes (gray bars) verified by RT-PCR (red bars) in mouse cortex (kindly provided by Marc Sultan and Marie-Laure Yaspo).[34] LOWESS, locally weighted polynomial regression

If several different experimental conditions are screened (for example different time points after medical treatment), then each gene expresses a certain numerical profile across these conditions. *Clustering algorithms* are explorative statistical methods that group together genes with similar profiles and separate genes with dissimilar profiles, whereby similarity (or dissimilarity) is defined numerically by a pairwise (dis)similarity function such as Euclidean distance or Pearson correlation.[37-40] Hierarchical clustering can be combined with a color-coded representation of the signal values (the expression patterns) and visualized in the form of a dendrogram. Clustering is a very intuitive way of visualizing data, but it should be pointed out that the dendrogram is strongly dependent on the parameters chosen for cluster analysis. Thus, each clustering process should undergo decent validation.[41] Associated groups of genes are usually further investigated, for example for common binding sites in the promoter sequences of the genes[42] or for common functional content.[43]

The major result of the explorative analysis is essentially a list of potential marker genes relevant for the disease or treatment under analysis. Since microarray data is error-prone, this list contains a lot of false positives. Thus, further filtering steps are commonly included in the analysis. Recent methods therefore aim at the correlation of the gene expression profiles with complementing sources of data such as pathway annotation, gene ontology (GO) categories, sequence analysis, clinical data, etc.[44-46]

Genes do not act as individual units; they collaborate in overlapping pathways, the deregulation of which is a hallmark for the disease under study. New bioinformatics tools have been developed that judge gene expression changes in the context of such *pathway analysis*. We have developed a method that judges the alteration of entire pathways with respect to two experimental conditions. This has been applied recently for the identification of pathways altered upon differentiation of inner cell mass and trophectoderm in the human blastocyst[47] and upon hormone-induced aging of the human skin.[48] The procedure is based on pathway annotation (for example provided by the Kyoto Encyclopedia of Genes and Genomes [KEGG] pathway database).[49] This information is then translated into a two-dimensional statistical test problem that involves Wilcoxon's signed rank sum test in order to compute a Z-score for each pathway that quantifies the degree of alteration across the different experimental conditions. The results of the pathway analysis in the latter study, for example, implicate the involvement of several metabolic pathways in the aging process, such as C21-steroid hormone metabolism, phospholipid degradation, prostaglandin and leukotriene metabolism, 2,4-dichlorobenzoate degradation, and fatty acid biosynthesis. Interestingly, pathways operative in neurodegenerative disease such as Huntington's disease (HD),[50,51] dentatorubral-pallidoluysian atrophy (DRPLA),[52] and amyotrophic lateral sclerosis (ALS)[53] also showed significant age-dependent expression changes.

Databases, standardization initiatives, and common platforms

It has been recognized that there is a fundamental need worldwide to share microarray data in order to correlate researchers' results with already published data. Since for such a task it is necessary to provide the raw data, large microarray databases have been set up as public repositories (for example the gene expression omnibus (GEO) from NCBI[54] and ArrayExpress from EBI[55]).

Functional annotation is provided by the GO consortium.[56] The aim of GO is to maintain a consistent, species-independent, functional description of gene products. GO terms have a defined parent-child relationship and form a directed acyclic graph (DAG). At the root of the GO are the three top-level categories—molecular function, biological process, and cellular component—which contain many levels of child nodes (GO terms) that describe a gene product with increasing specificity. There are several tools for mining these annotations. We have developed the GOblet server that computes GO-term graph annotation for DNA sequences comprising several different sequence databases.[57,58]

A particular data repository for neuroscience applications is the National Brain Databank, a publicly accessible gene expression repository for the collection and dissemination of results from postmortem studies of neurological and psychiatric disorders. The project has been developed by the Harvard Brain Tissue Resource Center (HBTRC) in collaboration with Akaza Research, as an online resource for the neuroscience community.

A further useful database for drug discovery and drug response screening is PharmGKB.[59,60] This database is a central repository for genetic, genomic, molecular, and cellular phenotype data and clinical information about people who have participated in pharmacogenomics research studies. The data includes, but is not limited to,

clinical and basic pharmacokinetic and pharmacogenomic research in the cardiovascular, pulmonary, cancer, pathway, metabolic, and transporter domains. Currently, information on 385 drugs and 22 different pathways can be reviewed.

Standardization and the development of standard operating procedures (SOPs), both for data generation and data analysis, are major issues in community initiatives. Whereas SOPs are widespread in experimental procedures, they are not available for the data-analysis part. Publications often report data analysis methods that are hard to reproduce. Thus, it has been worthwhile to develop some common analysis platforms. Besides commercial programs there have been powerful open-source platforms such as R/Bioconductor. These platforms contain standard statistical procedures, visualization methods, and methods for importing and exporting data. In a script-based language data analysis methods can be reported and easily reproduced.

The "druggable genome"

The detection of genes (or compounds) that have a particular molecular feature that makes them useful for measuring disease progression or effects of treatments can be enhanced by microarray analysis, provided there is a robust data analysis and correlation of the experimental outcome. Other functional genomics technologies are needed to complement the results obtained from microarrays. These technologies (such as proteomics, metabonomics, etc.) are inherent in standard drug screening workflows in pharmaceutical companies.[61] However, the flood of data produced is not easily handlable, and requires a new generation of computational tools that are more effective in managing the data and are able to embed the obtained result in a functional context.[62,63] Current treatments for most neurological disorders are either ineffective or minimally effective or produce severe side effects (for example antipsychotic medication in schizophrenia[64,65]). Thus, there is a clear need for better methods. A potential direction could be the development of compounds that effectively address the disease pathways driven by disease-related pathway identification methods.

It has been reported that the number of potential drug targets is fairly limited. An assessment of the number of genes that might serve as potential targets for drugs has been given recently.[66,67] Starting from the fact that there are well-known properties that define good drugs, the number of potential drug targets is predictable. These properties can be summarized as[68]:

- presence of more than five hydrogen-bond donors
- molecular mass >500 d
- high lipophilicity
- more than 10 nitrogen and oxygen atoms.

These properties increase the likelihood of oral bioavailability of a compound, ie, what makes it a commercially viable drug. Looking at the binding sites on human protein sequences for such compounds, only approximately 400 potential targets have been identified. Extending these targets to all members of their relevant gene families, approximately 3000 molecular targets can be identified. Most of these genes belong to a few gene families such as G protein coupled receptors (GPCRs), serine/threonine and tyrosine protein kinases, and nuclear hormone receptors. The implications of these estimations are that the limited number of druggable targets will be well explored within the next decade, with chemical leads being available for most of them. Thus, there will be a shift from the development of leads to the investigation of the molecular consequences of the drug treatment in the individual patient.

Challenges in neuroscience applications

Drug discovery and treatment in neuroscience face specific challenges, in particular regarding the availability of tissue, poor diagnosis, complexity of brain tissue, and the lack of good model systems for drug target validation.[69] Tissue samples in neuroscience applications are mostly post-mortem brain samples from affected individuals. These samples typically reflect the end stage of the disease, which highly biases the material and makes it impossible to study early disease stages.[70] Furthermore, the patients have typically undergone some disease treatment, which has an influence on the gene expression. Thus, separating the effects of these treatments from the effects of the disease is extremely difficult. Here, animal models and tissue culture systems can help to identify marker genes and pathways for the disease, as is common in other studies. For example, in a recent work we have utilized a mouse model (Ts65DN[71]) for trisomy 21 in order to identify genes that show dosage imbalances with respect to aneuploidy.[29] Results for many genes (such as *APP*) could be extrapolated to human tissue samples. Good animal models allow the extraction of untreated brain material as well as material from control samples.

Rodent and (particularly) nonhuman primate models are primarily interesting in this respect.

Current research utilizes microarrays in several areas of neuroscience research, such as schizophrenia,[72,73] brain cancer,[74] Alzheimer's disease (AD),[75] and HD.[76] These studies compare gene expression changes in patient and control groups, and show that microarrays are valuable tools for the expression profiling of drug response in human individuals.

Interestingly, the latter study incorporated blood samples from patients and control subjects and revealed changes in blood mRNAs that reflect disease mechanisms observed in HD brain. Moreover, these alterations correlate with disease progression. For example, they were able to identify genes altered in blood from HD patients (such as *ANXA*, *CAPZA1*, *HIF1A*, *P2Y5*, *SF3B1*, *SP3*, and *TAF7*) that were also differentially expressed in HD postmortem brain. This work implies the potential of using easily accessible tissue such as blood for monitoring the progression of complex brain disorders.

Systems biology as a new research paradigm

Systems biology aims at the explanation of physiology and disease from the level of interacting components such as molecular pathways, regulatory networks, cells, organs, and ultimately the entire organism.[77] With the use of computer models for such processes *in silico* predictions can be generated on the state of the disease or the effect of the individual therapy. The new approaches are about to revolutionize our knowledge of disease mechanisms and of the interpretation of data from high-throughput technologies.[1]

These approaches are necessary, considering the increasing complexity of research. Often, several laboratories are working with different techniques on the same problem. A fundamental challenge is thus to search through the exhaustive set of data and extract meaningful information. Here, *in silico* experiments can be the basis for a more successful drug screening.

Furthermore, there is a fundamental need for integration rules and methods. Multiple databases exist, a variety of experimental techniques have produced gene and proteome expression data from various tissues and samples, and important disease-relevant pathways have been investigated. Information on promoter regions and transcription factors is available for many genes as well as sequence information. This information—although extremely helpful—cannot be utilized in a sufficient way because of the lack of integrative analysis tools. A fundamental aim of systems biology is the understanding of the underlying biological processes on the basis of this data.

Crucial for the step from qualitative, explorative data analysis to quantitative, predictive analysis is the combination of experimental data with the knowledge of the underlying biological reaction system. This approach makes it possible to come up with conclusions about the properties of the system, even those that are not subject to experiments or are not even amenable by any experimental approach. For this purpose we have developed the modeling and simulation system PyBioS.[78] With this system it is possible to construct models that are based on the topology of a cellular reaction network and adequate reaction kinetics. Based on this information the system can automatically construct a mathematical model of differential equations that can be used for subsequent simulation of the temporal behavior and model analysis. Particularly, information on the topology of biological systems is available from several databases (eg, KEGG). PyBioS provides interfaces to these databases that can be used for the construction of appropriate model prototypes. Models include metabolic pathways, signal transduction pathways, transport processes, gene regulatory networks, among others, and can be accessed via a Web interface.

Mathematical models for disease pathways have been developed, predominantly for cancer. Examples are general emergence of properties of signaling pathways[79] such as extended signal duration, threshold behaviors, etc, endodermal growth factor receptor (EGFR) signaling,[80-82] and the TNF alpha-mediated NF-kappa B-signaling pathway (NFκB).[83,84] Specific pathway models for neuroscience applications are currently rare. Nevertheless, an understanding of the dynamics of these diseases could help to develop strategies to halt them at the stage they have reached at detection, or to prevent them entirely.[85]

Conclusion

Despite the great uncertainties inherent in functional genomics techniques, they will be indispensable for future work in drug development and therapy monitoring. However, these techniques must be accompanied by solid support from data analysis. Bioinformatics, and to an increasing degree, systems biology, have key roles in

this process. The information that we can gain about a biological system (for example a disease process) appears in practice as an experimental observation, and research is restricted to the targeted molecular level and the precision of the experimental techniques in use. It is very likely that the range of this experimental granularity will increase in the coming years, utilizing heterogeneous techniques that target a biological question of interest at different points so that data integration becomes a major challenge for future biomedical research.

In the case of complex disease conditions it is clear that such integrated approaches are required in order to link clinical, genetic, behavioral, and environmental data with diverse types of molecular phenotype information and to identify correlative associations. Such correlations, if found, are the key to identifying biomarkers and processes that are either causative or indicative of the disease.

In order to screen the success of drug treatment in the individual patient, new generations of tools and research methods will be developed. These tools will enable us to perform the crucial step from qualitative to quantitative analysis. Systems biology is pointing in this direction. With its close connection of experimental data generation, predictive data modeling, and subsequent validation it holds the promise of providing computational tools capable of personalized treatment and therapy monitoring in the individual patient. ❑

The authors wish to thank Christoph Wierling for proofreading the manuscript and Sylvia Krobitsch for providing neuroscience literature.

REFERENCES

1. Hood L, Perlmutter RM. The impact of systems approaches on biological problems in drug discovery. *Nat Biotechnol*. 2004;22:1215-1217.
2. Booth B, Zemmel R. Prospects for productivity. *Nat Rev Drug Discov*. 2004;3:451-456.
3. Weinshilboum R. Inheritance and drug response. *N Engl J Med*. 2003;348:529-537.
4. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*. 1999;286:531-537.
5. Gerhold DL, Jensen RV, Gullans SR. Better therapeutics through microarrays. *Nat Genet*. 2002;32:547-552.
6. Adler AS, Lin M, Horlings H, Nuyten DS, van de Vijver MJ, Chang HY. Genetic regulators of large-scale transcriptional signatures in cancer. *Nat Genet*. 2006;38:421-430.
7. Mischel PS, Cloughesy TF, Nelson SF. DNA-microarray analysis of brain cancer: Molecular classification for therapy. *Nat Rev Neurosci*. 2004;5:782-792.
8. Segal E, Friedman N, Kaminski N, Regev A, Koller D. From signatures to models: understanding cancer using microarrays. *Nat Genet*. 2005;37:S38-S45.
9. Lockhart DJ, Dong H, Byrne MC, et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol*. 1996;14:1675-1680.
10. Hughes T, Mao M, Jones A, et al. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat Biotechnol*. 2001;19:342-347.
11. Gunderson KL, Kruglyak S, Graige MS, et al. Decoding randomly ordered DNA arrays. *Genome Res*. 2004;14:870-877.
12. Kuhn K, Baker SC, Chudin E, et al. A novel high-performance random array platform for quantitative gene expression profiling. *Genome Res*. 2004;14:2347-2356.
13. Hardiman G. Microarray platforms – comparisons and contrasts. *Pharmacogenomics*. 2004;5:487-502.
14. Lehrach H, Drmanac R, Hoheisel J, et al. Hybridization fingerprinting in genome mapping and sequencing. In: Davis KE, Tilghman S, eds. *Genome Analysis: Genetic and Physical Mapping.* Cold Spring Harbor, NY; 1990:39-81.
15. Lennon G, Lehrach H. Hybridization analyses of arrayed cDNA libraries. *Trends Genet*. 1991;7:314-317.
16. Schena M, Shalon D, Davis R, Brown P. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*. 1995;270:467-470.
17. Adjaye J, Herwig R, Herrmann D, et al. Cross-species hybridisation of human and bovine orthologous genes on high density cDNA microarrays. *BMC Genomics*. 2004;5:83.
18. Newton SS, Bennett A, Duman RS. Production of custom microarrays for neuroscience research. *Methods*. 2005;37:238-246.
19. Parrish ML, Wei N, Duenwald S, et al. A microarray platform comparison for neuroscience applications. *J Neurosci Meth.* 2004;132:57-68.
20. Kuo WP, Jenssen TK, Butte AJ, Ohno-Machado L, Kohane IS. Analysis of matched mRNA measurements from two different microarray technologies. *Bioinformatics*. 2002;18:405-412.
21. Tan PK, Downey TJ, Spitznagel EL, et al. Evaluation of gene expression measurements from commercial microarray platforms. *Nucleic Acids Res*. 2003;31:5676-5684.
22. Barnes M, Freudenberg J, Thompson S, Aronow B, Pavlidis P. Experimental comparison and cross-validation of the Affymetrix and Illumina gene expression analysis platforms. *Nucleic Acids Res*. 2005;33:5914-5923.
23. Dai M, Wang P, Boyd AD, et al. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. *Nucleic Acids Res*. 2005;33:e175.
24. Jain AN, Tokuyasu TA, Snijders AM, Segraves R, Albertson DG, Pinkel D. Fully automatic quantification of microarray image data. *Genome Res*. 2002;12:325-332.
25. Wierling CK, Steinfath M, Elge T, et al. Simulation of DNA array hybridization experiments and evaluation of critical parameters during subsequent image and data analysis. *BMC Bioinformatics*. 2002;3:29.
26. Steinfath M, Wruck W, Seidel H, Lehrach H, Radelof U, O'Brien J. Automated image analysis for array hybridization experiments. *Bioinformatics*. 2001;17:634-641.
27. Holleman A, Cheok MH, denBoer ML, et al. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. *N Engl J Med*. 2004;351:533-542.
28. Quakenbush, J. Microarray data normalization and transformation. *Nat Genet*. 2002;496-501.
29. Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *J Am Stat Assoc*. 1979;74:829-836.
30. Cleveland WS, Devlin SJ. Locally weighted regression: an approach to regression analysis by local fitting. *J Am Stat Assoc*. 1983:83:596-610.
31. Yang H, Dudoit S, Luu P, et al. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variations. *Nucleic Acids Res*. 2002;30:e15.
32. Li C, Wong, WH. Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. *Proc Natl Acad Sci U S A.* 2001:98:31-36.

El perfil de expresión de la respuesta a los fármacos: desde los genes a las vías involucradas

La comprensión de la respuesta individual a un fármaco –que determina su eficacia y tolerabilidad en el organismo– es el principal cuello de botella en el desarrollo actual de fármacos y ensayos clínicos. La respuesta intracelular y el metabolismo, donde participan por ejemplo las enzimas del citocromo P-450, puede aumentar o disminuir el efecto de diferentes fármacos, dependiendo de la variante genética. La tecnología de microarrays ofrece el potencial para mapear la composición genética del paciente individual. Sin embargo, como los experimentos no son tan precisos deben acompañarse de un análisis sólido y consistente de los datos. Además, la investigación reciente apunta a la combinación de datos con metodología proveniente de modelos matemáticos que permiten una asistencia orientada a problemas en el proceso de descubrimiento de fármacos. Este artículo revisará el estado actual del conocimiento acerca de las plataformas de tecnología de arrays de ADN y los elementos básicos del análisis de los datos y la investigación bioinformática en el descubrimiento de fármacos. Para incrementar el análisis de un gen único, se presentará un nuevo método para la interpretación de los cambios en la expresión de los genes teniendo en cuenta todas las vías involucradas. Además se introducirá el concepto de biología de sistemas, como un nuevo paradigma para el desarrollo de fármacos, y se destacará nuestra reciente investigación acerca del desarrollo de un modelo y una plataforma de simulación para aplicaciones biomédicas. Finalmente se discutirán las potencialidades de la biología de sistemas para los modelos de respuesta a fármacos en el paciente individual.

Profilage de l'expression de la réponse au médicament : des gènes aux voies d'accès

La compréhension de la réponse individuelle au médicament, ce qui détermine son efficacité et sa tolérance chez le patient, est le principal goulet d'étranglement des essais cliniques et du développement des médicaments actuels. Le métabolisme et la réponse intracellulaires, par exemple à travers les enzymes du cytochrome P-450, peut soit augmenter soit diminuer l'effet des différents médicaments, selon la génétique. Des microéchantillons (microarrays) permettent de déterminer la configuration génétique de chaque patient. Ces techniques sont toutefois imprécises, justifiant une méthodologie précise et exigeante lors de l'analyse. De plus, la recherche récente permet un débit élevé de données avec des méthodes de modèlisation mathématique permettant de résoudre les problèmes ayant trait aux moyens de découverte des médicaments. Cet article concerne les techniques de pointe des plates-formes de technologie de microéchantillons d'ADN ainsi que les bases de l'analyse de données et de la recherche bio-informatique pour la découverte des médicaments. Nous présenterons une nouvelle méthode consistant à décrire les modifications de l'expression génétique au niveau de toute une cascade de réponses biologiques. Nous introduirons le concept de biologie des systèmes comme un nouveau paradigme pour le développement des médicaments et nous mettrons l'accent sur notre recherche récente, le développement d'une plate-forme de simulation et de modélisation pour les applications biomédicales. Nous discuterons du potentiel de la biologie des systèmes pour la modélisation de la réponse de chaque patient au médicament.

33. Irizarry RA, Bolstad BM, Collins F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res*. 2003;31:e15.

34. Draghici S. *Data Analysis Tools for DNA Microarrays*. Boca Raton, Fla: Chapman & Hall/CRC Press; 2003.

35. Kahlem P, Sultan M, Herwig R, et al. Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of Down syndrome. *Genome Res.* 2004;14:1258-1267.

36. Herwig R, Aanstad P, Clark M, Lehrach H. Statistical evaluation of differential expression on cDNA nylon arrays with replicated experiments. *Nucleic Acids Res.* 2001;29:E117.

37. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A*. 1998;95:14863-14868.

38. Tamayo P, Slonim D, Mesirov J, et al. Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. *Proc Natl Acad Sci U S A.* 1998;96:2907-2912.

39. Herwig R, Poustka AJ, Müller C, Lehrach H, O'Brien J. Large-scale clustering of cDNA fingerprinting data. *Genome Res*. 1999;9:1093-1105.

40. Sharan R, Shamir R. CLICK: a clustering algorithm with applications to gene expression analysis. Paper presented at: Proceedings of the 8th International Conference on Intelligent Systems for Molecular Biology (ISMB); 2000; Menlo Park, California, USA.

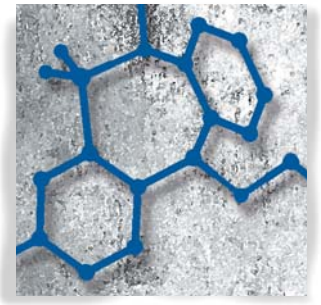
41. Jain AK, Dubes RC. *Algorithms for Clustering Data*. Englewood Cliffs, NJ: Prentice Hall;1988.

42. Tavazoie S, Hughes JD, Campbell MJ, Cho RJ, Church GM. Systematic determination of genetic network architecture. *Nat Genet*. 1999;22:281-285.

43. Gibbons FD, Roth FP. Judging the quality of gene expression-based clustering methods using gene annotation. *Genome Res*. 2002;12:1574-1581
44. Gitton, Y, Dahmane, N, Baik, S, et al. A gene expression map of human chromosome 21 orthologues in the mouse. *Nature*. 2002; 420:586-590.
45. Rhodes DR, Barrette T, Rubin MA, Ghosh D, Chinnaiyan AM. Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. *Cancer Res*. 2002;62:4427-4433.
46. Rhodes DR, Yu J, Shanker K, et al. Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression. *Proc Natl Acad Sci U S A.* 2004;101:9309–9314.
47. Adjaye J, Huntriss J, Herwig R, et al. Primary differentiation in the human blastocyst: Comparative molecular portraits of inner cell mass and trophectoderm cells. *Stem Cells*. 2005;23:1514-1525.
48. Makrantonaki E, Adjaye J, Herwig R, et al. Signalling and metabolic pathways associated with hormone-induced aging in human sebocyte cells in vitro. *Aging Cell*. 2006;5:331-344.
49. Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resources for deciphering the genome. *Nucleic Acids Res*. 2004;32:D277-D280.
50. Luthi-Carter R, Strand AD, Peters NL, et al. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum Mol Genet*. 2000;9:1259-1271.
51. Sipione S, Rigamonti D, Valenza M, et al. Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. *Hum Mol Genet*. 2002;11:1953-1965.
52. Luthi-Carter R, Hanson SA, Strand AD, et al. Polyglutamine and transcription: gene expression changes shared by DRPLA and Huntington's disease mouse models reveal context-independent effects. *Hum Mol Genet*. 2002;11:1927-1937.
53. Jiang YM, Yamamoto M, Kobayashi Y, et al. Gene expression profile of spinal motor neurons in sporadic amyotrophic lateral sclerosis. *Ann Neurol*. 2005;57:236-251.
54. Barrett T, Suzek TO, Troup DB, et al. NCBI GEO: mining millions of expression profiles—database and tools. *Nucleic Acids Res*. 2005;33(Database Issue):D562-D566.
55. Parkinson H, Sarkans U, Shojatalab M, et al. ArrayExpress—a public repository for microarray gene expression data at the EBI. *Nucleic Acids Res*. 2005;33(Database Issue):D553-555.
56. Gene Ontology Consortium. The gene ontology (GO) project in 2006. *Nucleic Acids Res*. 2006;34(Database Issue):D322-D326.
57. Hennig S, Groth D, Lehrach H. Automated Gene Ontology annotation for anonymous sequence data. *Nucleic Acids Res*. 2003;31:3712-3715.
58. Groth D, Lehrach H, Hennig S. GOblet: a platform for Gene Ontology annotation of anonymous sequence data. *Nucleic Acids Res*. 2004;32: W313-317.
59. Hewett M, Oliver DE, Rubin DL, et al. PharmGKB: the pharmacogenetics knowledge base. *Nucleic Acids Res*. 2002;30:163-165.
60. Thorn CF, Klein TE, Altman RB. PharmGKB: the pharmacogenetics knowledge base. *Methods Mol Biol*. 2005;311:179-191.
61. Kramer R, Cohen D. Functional genomics to new drug targets. *Nat Rev Drug Discov*. 2004;3:965-972.
62. Kanehisa M, Bork P. Bioinformatics in the post-sequence era. *Nat Genet*. 2003;33:305-310.
63. Dobrin SE, Stephan DA. Integrating microarrays into disease-gene identification strategies. *Expert Rev Mol Diagn*. 2003;3:375-385.
64. Dunckley T, Coon KD, Stephan DA. Discovery and development of biomarkers of neurological disease. *Drug Discov Today*. 2005;10:326-334.
65. Evans WE, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N Engl J Med*. 2003;348:538-549.
66. Hopkins AL, Groom CR. The druggable genome. *Nat Rev Drug Disc*. 2002;1:727-730.
67. Russ AP, Lampel S. The druggable genome: an update. *Drug Discov Today.* 2005;10:1607-1610.
68. Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 1997;23:2-25.
69. Mirnics K, Pevsner J. Progress in the use of microarray technology to study the neurobiology of disease. *Nat Neurosci*. 2004;5:434-439.
70. Mirnics K, Middleton FA, Lewis DA, Levitt P. Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. *Trends Neurosci*. 2001;24:479-486.
71. Reeves RH, Irving NG, Moran TH, et al. A mouse model for Down syndrome exhibits learning and behaviour deficits. *Nat Genet*. 1995;11:177-184.
72. Mirnics K, Middleton FA, Marquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron*. 2000;28:53-67.
73. Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P. Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *J Neurosci*. 2002;22:2718-2729.
74. Mischel PS, Cloughesy TF, Nelson SF. DNA microarray analysis of brain cancer: molecular classification for therapy. *Nat Rev Neurosci*. 2004;5:782-792.
75. Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW. Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor response. *Proc Natl Acad Sci U S A.* 2004;101:2173-2178.
76. Borovecki F, Lovrecic L, Zhou J, et al. Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease. *Proc Natl Acad Sci U S A*. 2005;102:11023-11028.
77. Butcher E, Berg EL, Kunkel EJ. Systems biology in drug discovery. *Nat Biotechnol*. 2004;22:1253-1259.
78. Klipp E, Herwig R, Kowald A, Wierling C, Lehrach H. *Systems Biology in Practice*. Weinheim, Germany: Wiley-VCH; 2005.
79. Bhalla US, Iyengar R. Emergent properties of networks of biological signaling pathways. *Science*. 1999;283:381-387.
80. Wiley HS, Shvartsman SY, Lauffenburger DA. Computational modeling of the EGF-receptor system: a paradigm for systems biology. *Trends Cell Biol*. 2003;13:43-50.
81. Schoeberl B, Eichler-Jonsson C, Gilles ED, Muller G. Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. *Nat Biotechnol*. 2002;20:370-375.
82. Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol Sys Biol*. 2005;1:2005.0010 Epub 2005 May 25.
83. Cho KH, Shin SY, Lee HW, Wolkenhauer O. Investigations into the analysis and modeling of the TNF alpha-mediated NF-kappa B-signaling pathway. *Genome Res*. 2003;13:2413-2422.
84. Hoffmann A, Levchenko A, Scott ML, Baltimore D. The I-kappaB-NF-kappaB signaling module: temporal control and selective gene activation. *Science*. 2005;298:1241-1245.
85. Aßmus HE, Herwig R, Cho KH, Wolkenhauer O. Understanding the dynamics of biological systems: roles in medical research. *Expert Rev Mol Diagn*. 2006. In press.

New directions for drug discovery

Michael Spedding, PhD



Modern drug discovery demands an integrative approach, using many different technologies, but ultimately based on an understanding of the pathophysiology of the disease state to be treated. Targeting drugs at the main pathophysiological process is the key to success. This issue needs to be addressed with the multiple screening systems available, which can be used to find new leads.

Dialogues Clin Neurosci. 2006;8:295-301.

Keywords: *drug discovery; pathophysiology; drug testing; screening*

Author affiliations: Deputy Research Director, Chairman NC-IUPHAR, Experimental Sciences, Servier Research Institute, Suresnes, France

Address for correspondence: Prof Michael Spedding, Deputy Research Director, Chairman NC-IUPHAR, Experimental Sciences, Servier Research Institute, 11 Rue des Moulineaux, 92150 Suresnes, France
(e-mail: michael.spedding@fr.netgrs.com)

The discovery and development of one new drug costs around $800 million (taking failures into account) and takes an average of 10 to 12 years. This degree of investment, with such a late return on this investment, is unparalleled in human activity.

Despite this investment, some areas of great therapeutic need do not have optimal treatments—acute stroke and Alzheimer's disease, as well as other central nervous system (CNS) disorders. There are no drugs registered for the treatment of acute stroke, which is an area of great therapeutic need, being the third-highest cause of mortality and the second-highest cause of morbidity. Nevertheless, there are distinct methodological reasons in the clinical trials which can preclude demonstrating efficacy in stroke under many circumstances.[1] Another area in which the pharmaceutical industry has failed to revolutionize therapy has been in the treatment of Alzheimer's disease. However, preventive therapy by addressing hypertension using angiotensin-converting enzyme inhibitors (perindopril, in the PROGRESS study) has shown marked reduction in the incidence of stroke, and also of dementia and cognitive decline.[2,3] Antidepressant drugs with higher efficacy and fewer side effects are much needed.

Effective drug discovery requires drug targets for therapeutic intervention which are pivotal points for the disease process, and up until now these have not been clearly identified for stroke (with the possible exception of tissue plasminogen activator for very early intervention) or Alzheimer's disease.

Background

Only 35 new compounds were registered with the Food and Drug Administration (FDA) in 2003 despite a research expenditure by the major pharmaceutical firms

of 33 billion dollars *(Figure 1)*. Part of these costs are due to the costs of failure. *Figure 2* shows the fate of a sample of 121 drugs put into phase 1 clinical trials by British pharmaceutical companies. The results are edifying. Although some drugs failed because of toxicological problems or metabolic issues, or were even stopped for commercial reasons, the major reason for failure was lack of efficacy. The drugs were stopped because they did not work. This may occur for several reasons.

First, the original hypothesis may be wrong, and the end result is a useful experiment, albeit a very expensive one.

Second, and this is perhaps just as likely, the animal models may not represent the tests used in phase I and phase II clinical trials—it is also possible that the tests used in phase I and II do not represent the true patient response.

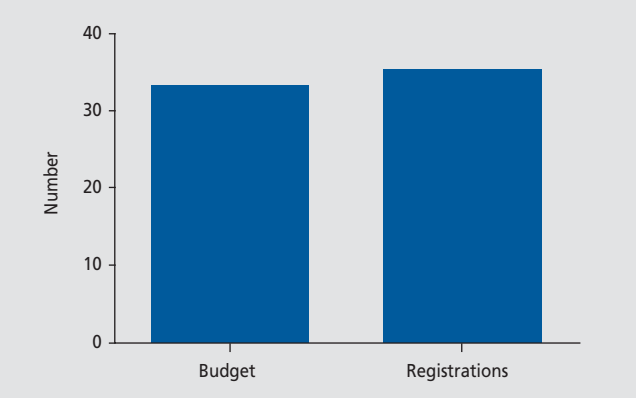


Figure 1. Research budget (billion $) and total number of US drug registrations in 2003.

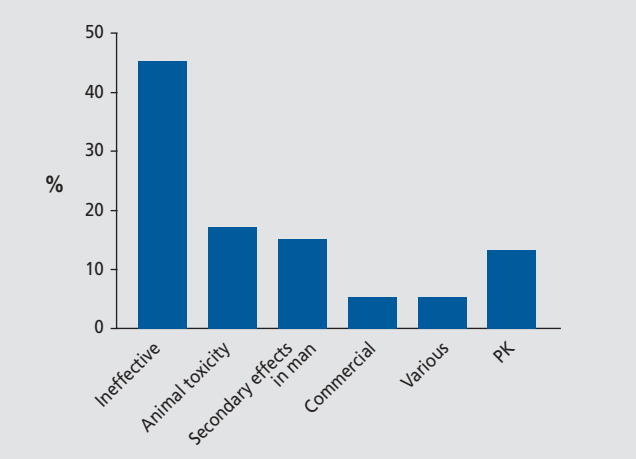


Figure 2. Reasons for stopping clinical development of 121 compounds in clinical trials carried out by seven British companies. PK, pharmacokinetics

Indeed, of the 340 compounds entering phase I per year, four out of five fail, and even when registration is achieved, less than half of the compounds recoup their development costs. The failure of drugs to work in the clinical setting (lack of efficacy due either to the concept not working, or to the animal models or the clinical models not responding to the patients' needs) is a key area for improvement.

Third, increasing safety requirements discourages risk. This is particularly the case for CNS-active drugs which may have cardiovascular side effects (effects on electrocardiographic [ECG] QTc intervals for example). It remains a truism that no drug can be effective without having some measure of risk.

However, it is now possible to have high-throughput screens for safety, and to do a better job of selecting compounds at an early stage.

The difficulties faced by a drug discoverer are shown by the sequence below. First, he or she must find the optimal structure/activity, then exclude structure/activity at other sites:

1. Definition of structure/activity at site of action
2. Exclusion of structure/activity at cytochromes
3. Exclusion of structure/activity—mutagenicity
4. Exclusion of structure/activity—cardiac QTc
5. Start of toxicity studies.

Fourth, there is the realization of the increasing complexity of biological systems. Although there may be only 25 000 to 30 000 genes, many of which are drug targets *(Figure 3)*, the gene products are much more complex because of alternative splicing, mRNA editing, receptor dimerization, functional trafficking (where drugs acting at the same receptor may have different effects) and the multiple post-translational controls and accessory proteins.

New technological opportunities

In vitro screening

Screening on recombinant proteins has proven to be immensely powerful, and can provide new leads from high-throughput screening on a scale which would be impossible with other technologies. Now the target proteins may even be crystallized, with the drug, or even with fragments of the drug, and the crystals analyzed to define the conformational changes induced in the target by different drugs. The throughput of this technology is such that entire chemical series can be analyzed for their direct effects on the protein of interest. Thus, hundreds

of thousands of compounds can be tested at the cellular target in a few months, and the "hits" can then be chemically optimized to make new metabolically stable drugs. *(Figure 3)*. Different conformational states during cellular activation, particularly in the presence of accessory proteins, may easily change a singe hydrogen bond or electrostatic attraction, changing affinity. Indeed, it must be pointed out that one additional hydrogen bond between the compound and the target can change the affinity thirty-fold. This complexity may induce inadequate responses to predict therapeutic efficacy. As compound selection is the crucial issue, we have argued that, after preliminary screens in recombinant systems, and following exclusion of inappropriate compounds (for metabolic or safety reasons), the selection of the final compound to proceed onto development should take place in pathophysiological models, and preferably, if breakthrough compounds are looked for, in *novel* pathophysiological models. However, this means a major investment in screening in animal models.

In vivo screening

Animal models are often the limiting factor in research (particularly for cognitive issues), and finding staff skilled in their handling is not easy. Previous drugs have been tested for in the established models, and the way to test benzodiazepine anxiolytics is to use the classic anxiety screening models, defined by diazepam. However, novel drugs working in new ways may need new models. Thus, compounds should be selected using a model of pathophysiological conditions. However, this needs skilled pharmacologists[4] with an integrative vision of pathophysiology.

How are new drugs discovered?

New drugs may be discovered in very many ways, but discovery nearly always involves tight collaborations between chemists and pharmacologists, who must identify the cellular and genetic factors important in pathophysiology, produce appropriate hypotheses, and design new test systems. Screening new molecules can be done in a number of ways.

Target identification

Ideally, the target should be the cause of a specific disease which can be targeted on a molecular level. There has been immense progress made in defining the receptor systems in the human genome, by analogy to existing 7-transmembrane receptors. This marks a unique moment in science, because many targets are becoming known. Lists of these receptors have been produced (eg, ref 5). Furthermore, new targets remain to be discovered, and the existing targets are known to have many different forms (alternative splicing, messenger ribonucleic acid (mRNA) editing, single-nucleotide polymorphisms, etc) which may allow selective targeting of disease states. The bioinformatics industry provides an immensely powerful tool to scientists, and many of these data are in the public domain.

Target validation

A crucial issue is to validate the target, in animal and preferably in human models. This is critical, because of the high cost of discovering a new drug for a target and performing the clinical experiment to find out whether the new drug works in a disease state in man. As there are tens of thousands of potential targets, target validation is a crucial issue. Fortunately, transgenic models may help in this regard, but their predictivity is only relative.

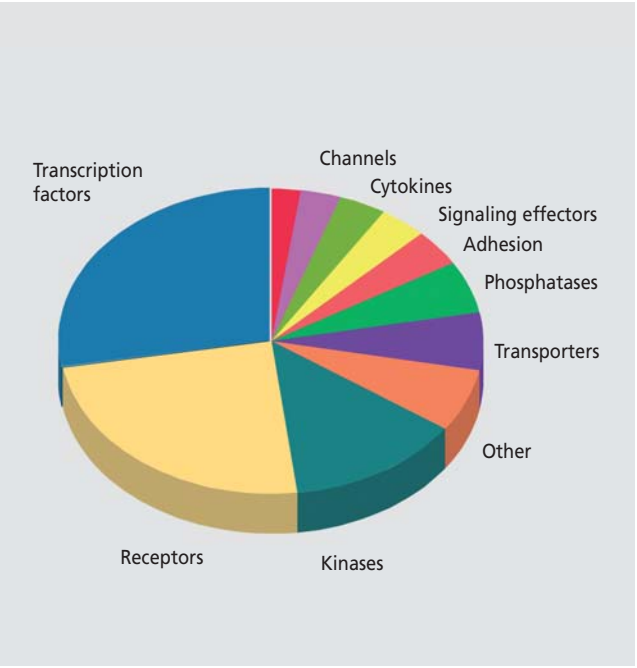


Figure 3. Signaling genes in the human genome.

Lead identification

A lead compound is usually selected by high-throughput screening of compound collections, or libraries *(Figure 4)*. These compound libraries may consist of thousands, or hundreds of thousands, of compounds, built up by the pharmaceutical company over the years. Virtual screens can now be performed by modeling the interactions of the target with virtual libraries consisting of all the compounds which are commercially available—the best compounds can then be selected for screening. The "hits" or compounds which are active in the first round of screening are then optimized so that they possess the properties needed in a new drug. Testing is then done on each of these molecules to confirm its effect on the drug target.

Lead optimization

Lead optimization compares the properties of various lead compounds, allowing selection of the compound or compounds with the greatest potential to be developed into safe and effective medicines. The metabolism is optimized in high-throughput screens to produce compounds which retain their activity at the target of interest, while being metabolically stable and well absorbed.

Drug testing in humans

Testing an investigational new drug requires submission of all the information about the drug for permission to administer to healthy volunteers or patients. Not only

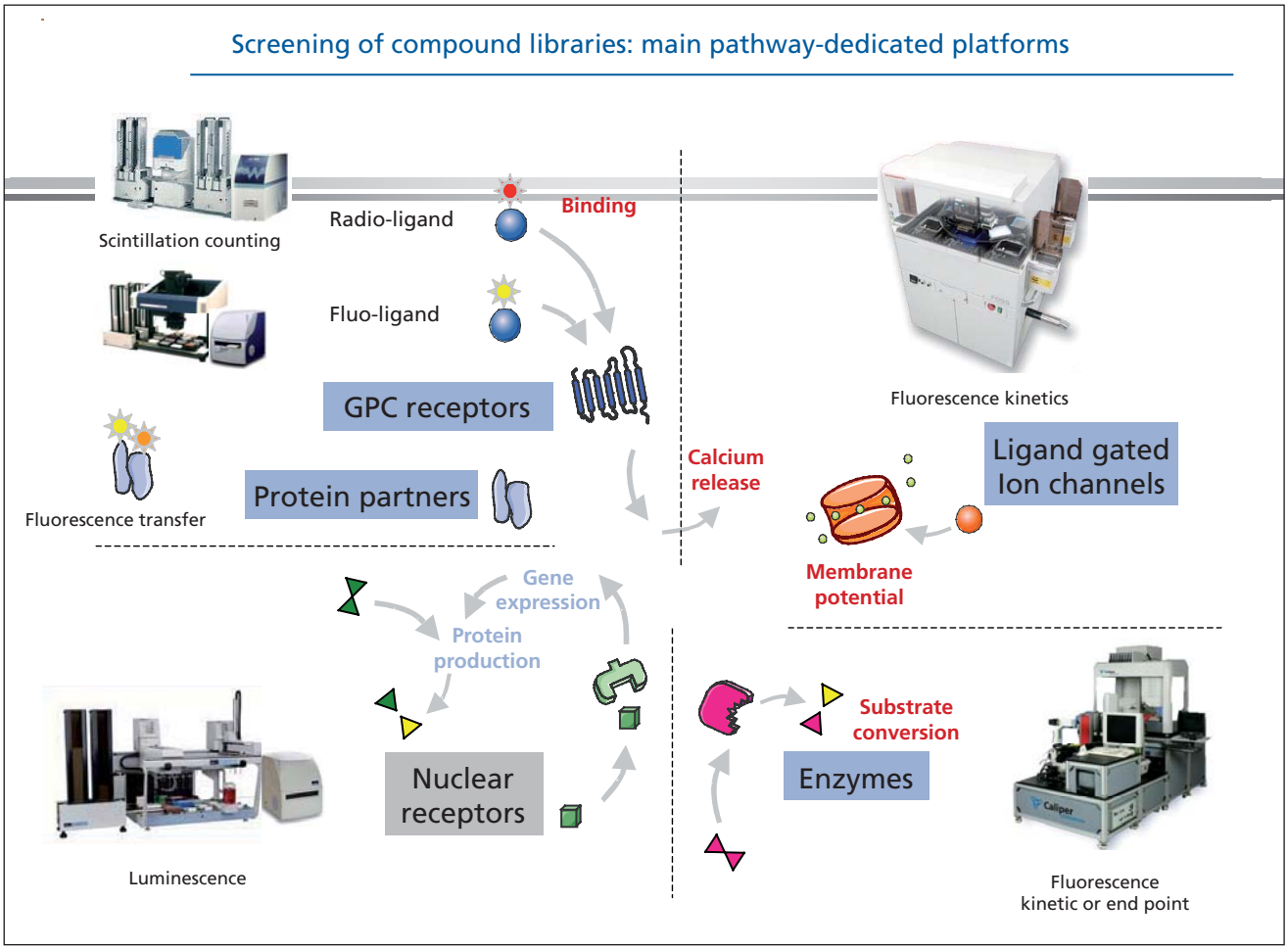


Figure 4. Screening of compound libraries: main pathway-dedicated platforms. GPC, G protein-coupled (Figure courtesy of Olivier Nosjean and Jean Boutin, Servier research).

regulatory authorities, but also institutional or independent review boards (IRB) or ethical advisory boards approve the experimental protocol, well as the informed consent documents signed by the volunteers.
The clinical testing of the drug passes through Phase I, Phase II, and Phase III clinical studies. In each successive phase, increasing numbers of patients are tested, but the success or failure of the drug *(see Figure 2)* depends not only on its mode of action, but also on the good methodological quality of the testing schedule used in the clinic.

Phase I clinical studies

Phase I studies must verify the safety and tolerability of the new drug in volunteers, showing the maximal tolerated dose, and how it is absorbed, distributed, metabolized, and excreted. This phase takes 6 months to a year. Healthy volunteers are administered the drug acutely and then chronically. The hypothesis of action may be tested pharmacologically with indexes of brain penetration, brain imaging, and electroencephalogram (EEG). However, it must be borne in mind that healthy individuals may not react in the same way as patients. Some drugs cannot be tested in healthy volunteers (eg, in oncology).

Phase II clinical studies

Phase II studies are a critical research area designed to show effectiveness, define dose-response for the critical phase III approval studies, and demonstrate a measure of safety in the patient population. This phase of development generally takes from 1 to 3 years with several hundred patients. It is here that an appropriate choice of drug effectiveness criteria for drug effectiveness, linked to animal models, yet providing a realistic test of the drug in the patient population, can make a real difference.

Phase III clinical studies

Phase III studies show effectiveness and safety in randomized and blinded clinical trials involving thousands of patients. This phase can take 2 to 5 years, and is the most expensive clinical testing phase.

New Drug Application/Marketing Authorization

A New Drug Application (NDA), in the US, or Marketing Authorization (MA), in Europe, documents the safety and efficacy of the proposed drug, and the applications contain all the reports from the drug development process. At the end of phase III, the evidence proving efficacy and safety are submitted. The approval process can take 1 to 2 years, followed by post-marketing surveillance and extension of the therapeutic indications and patient populations.

Fast-tracking

Several regulatory issues may be seen as opportunities. Fast tracking for very urgent therapeutic needs, such as treatment for acquired immune deficiency syndrome (AIDS), has been introduced by the FDA. Furthermore, the FDA have issued guidelines on pharmacogenetic subtyping of patient populations (responders, patients at risk for side effects, rapid metabolizers, etc).

Partnership

Modern drug discovery and development depends on a constant partnership between the actors in the project, in the many disciplines which are involved. The partnership between industry and academia is a critical issue, because basic research can lead to many unexpected breakthroughs, of which the researcher may not appreciate the industrial and medical importance. It is correct that financial return should be associated with inventiveness. However, the fewer industrial partners there are (as in France), the fewer local industrial partners there are for startup biotechnology companies. There is thus a delicate balance between support of pharmaceutical companies and small biotechnology companies. As the main industrial experience (to avoid the pitfalls shown in *Figure 2* for example) is located in pharmaceutical companies, this pragmatic feedback and review is an essential part of the health of the local industrial environment. It is also essential that research remains very medically oriented, because the patients' needs come first. Partnership with clinicians and top medical teams is therefore also a key element for success. However, all of the stages of drug discovery remain an experiment, and must be designed as such. After the initial selection process which finds the drug, the only thing which does not change in the development process is the molecule; all the others—the scientists, sometimes even the therapeutic area—may change. However, the molecule can do no more or less than on the day when it is chosen, which is why the tests which select the molecule are so important.

Table I shows the factors influencing success in the drug discovery process.

• 1. Resources. Resources are critical, and all programs must have access to major molecular resources for screening for drug discovery.
• 2. Access to a rapid drug development program, with clinical tests which are tightly related to the animal tests. Thus, feedback to discovery scientists can be rapid to optimize discovery and to ensure that researchers do not continue to work on hypotheses which do not stand up in the clinic.
• 3. Approaches and strategies. This is a critical issue for success, and requires medicalized research, in association with the specialized resources. Reverse engineering animal models from our new knowledge of brain systems in disease states will better reflect pathophysiological situations and allow selection of new drugs.
• 4. Quality/creativity. The quality, application, and creativity of the scientists involved, together with the relevance of the approaches and strategies, are key factors for success. However, there are few measures, other than past records, which can be quantified by objective criteria (other than publications, but these are not always related to drug discovery capacity).
• 5. Outside research. Having access to a large network of academic contacts is crucial to increasing the number of ideas processed and increasing throughput.

Table I. What are the main factors influencing success in drug discovery processes, and how can research output be improved?

Key points for definition of new ways forward in psychiatric disorders

It is important to define the specific nodes or switch-points which are modified by disease processes and suitable for therapeutic intervention. These can be at several levels, such as:

1. *Molecular*—the multiple intracellular signaling cascades have key nodal points which can be targetted. Cancer drugs are targetted at key points, and now the same situation is being extended into CNS research, where drugs for bipolar disorder, such as lithium, may interact with key signaling molecules such as glycogen synthase kinase 3 (GSK3).
2. *Epigenetic changes* where the genes expressed relate to the past history of the idividual. Furthermore, many gene products are modified by alternative splicing or mRNA editing which can change the function of key proteins in pathophysiological conditions.
3. *Cell plasticity*. Neurotrophins and cytokines have major effects on cell plasticity and integrity. Many genes can interact within the neurotrophic signaling cascades, and these are major points for therapeutic interventions. For example, we have shown that brain-derived neurotrophic factor (BDNF), the key neurotrophin involved in activity-dependent resculpting of neuronal networks, can also change the respiratory coupling efficiency of mitochondria, indicating a new way forward in the links between cellular activity and coupled metabolism.[6]
4. The *neurotransmitters* involved in modulating brain systems are well defined, and still represent sources of drug discovery (noradrenaline, 5-HT, dopamine, etc). However, the multiple states of receptors and their signaling pathways warn against oversimplification.[7]
5. *Chronobiological issues* are important in resetting biological rhythms, and may be even more important than previously thought. The finding that agomelatine, a melatonin agonist and 5-HT_{2C} antagonist, can be an effective antidepressant with a low side-effect potential[8,9] reconfirms the interest in chronobiological systems, because their dysreg-

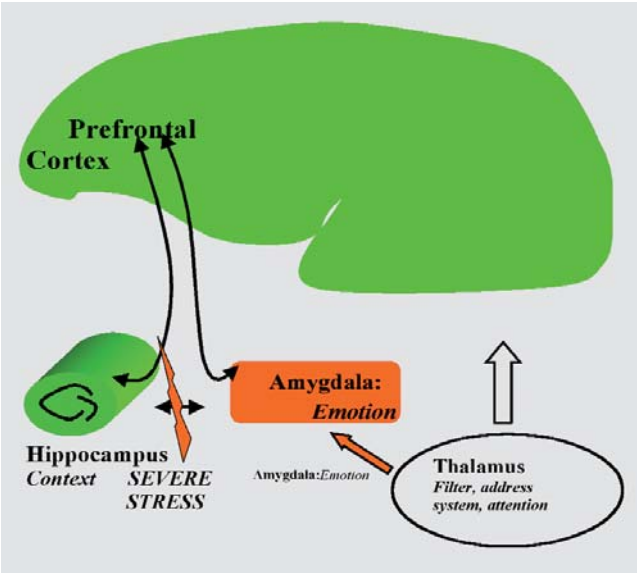


Figure 5. The impact of stress on neuroplasticity may be a novel target for drugs in psychiatry, as stress inhibits plasticity in hippocampal and prefrontal cortex circuits while increasing plasticity in the circuits dealing with emotion (amygdala, prefrontal cortex).[10]

ulation is a common feature of ageing and psychiatric disorders.

6. *Cell firing on specific nodal points*. The systems in the brain are becoming well defined, and it is now possible to intervene on brain switch-points, which may be deregulated. These can be quantified electrophysiologically, or by microdialysis of the main neurotransmitters, or by brain imaging techniques.
7. *Neuronal networks* for brain functions (eg, the main systems involved in cognition, decision, and emotivity and fear (prefrontal cortex, amygdala, hippocampus, *Figure 5*). An example of research in this area is the finding that stress blocks long-term potentiation (LTP, a measure of plasticity) in the hippocampal to ventromedial prefrontal cortex,[11] and these effects are reversed acutely by an atypical antidepressant, tianeptine. McEwen's group have shown that these acute effects change into effects on dendritic arborization.[12] Furthermore, there is now proof of concept that this pathway is of critical importance for depression because Mayberg's group[13] have implanted electrodes into the white matter behind Cg25 (the equivalent in man of the ventromedial prefrontal cortex in rodents) and found immediate antidepressant effects in patients who had been entirely treatment-resistant. Targetting these brain areas therefore opens up new perspectives in drug discovery for depression. Furthermore, reingineering animal models to study these brain areas will allow the selection of new classes of molecule. ❑

REFERENCES

1. Spedding M. Reasons why stroke trials underestimate the neuroprotective effects of drugs. *Stroke*. 2002;33,324-325.
2. The PROGRESS Collaborative Group. Effects of blood pressure lowering with perindopril and indapamide therapy on dementia and cognitive decline in patients with cerebrovascular disease. *Arch Intern Med*. 2003:163:1069-1075.
3. Dufouil C, Chalmers J, Coskun O, et al. Effects of blood pressure lowering on cerebral white matter hyperintensities in patients with stroke. *Circulation*. 2005;112:1644-1650.
4. Collis MG. Integrative pharmacology and drug discovery - is the tide turning? *Nature Drug Discovery*. 2006:5,377-379.
5. Foord SM, Bonner TI, Neubig RR, et al. International Union of Pharmacology. XLVI. G protein-coupled receptor list. *Pharmacol Rev*. 2005;2:279-288.
6. Markham A, Cameron I, Franklin P, Spedding M. BDNF increases mitochondrial respiratory coupling, and glutamate metabolism, at complex I, but not complex II. *Eur J Neurosci*. 2004;20:1189-1196.
7. Kenakin T. New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nature Drug Discovery*. 2005;4:919-927.
8. Loo H, Hale A, D'haenen H. Determination of the dose of agomelatine, a melatoninergic agonist and selective 5-HT2c antagonist, in the treatment of major depressive disorder: a placebo-controlled dose range study. *Int Clin Psychopharmacol*. 2002;17:239-247.
9. Kennedy SH, Emsley R. Placebo-controlled trial of agomelatine in the treatment of major depressive disorder. *Eur Neuropsychopharmacol*. 2006;16:93-100.
10. Spedding M, Jay T, Costa e Silva J, Perret L. A pathophysiological paradigm for the therapy of psychiatric disease. *Nature Drug Discovery*. 2005;4:467-476.
11. Rocher C, Spedding M, Munoz C, Jay T. Acute stress-induced changes in cortical synaptic plasticity: interactions with antidepressants. *Cereb Cortex*. 2004;14, 224-229.
12. McEwen BS, Olié JP. Neurobiology of mood, anxiety and emotions, as revealed by studies of a unique antidepressant: tianeptine. *Mol Psych*. 2005;10:525-537.
13. Mayberg HS, Lozano A, Voon V, et al. Deep brain stimulation for treatment resistant depression. *Neuron*. 2005;45:651-660.

Nuevos caminos para el descubrimiento de fármacos

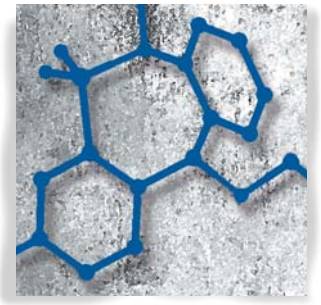
El descubrimiento moderno de fármacos requiere de una aproximación integradora en que se utilizan diversas tecnologías, pero en último término se basa en la comprensión de la fisiopatología del curso clínico de la enfermedad para que así pueda ser tratada. La clave del éxito está en conseguir fármacos que apunten al proceso fisiopatológico central. Este tema necesita ser abordado con los múltiples sistemas de exploración disponibles, los que pueden utilizarse para encontrar nuevas posibles moléculas.

Nouvelles directions dans la découverte des médicaments

La découverte moderne de médicaments demande une approche intégrée, utilisant des technologies variées; elle est fondée sur la compréhension de la physiopathologie de la maladie à traiter. La clé du succès est de cibler les médicaments qui agissent sur le principal processus physiopathologique. Cette question doit être abordée à l'aide des nombreux systèmes de sélection des molécules existants afin de trouver de nouvelles pistes.

Contributions of molecular biology to antipsychotic drug discovery: promises fulfilled or unfulfilled?

Bryan L. Roth, MD, PhD



This review summarizes the various conceptual paradigms for treating schizophrenia, and indicates how molecular biology and drug discovery technologies can accelerate the development of new medications. As yet, there is no convincing data that a crucial druggable molecular target exists which, if targeted, would yield medications with efficacies greater than any currently available. It is suggested, instead, that drugs which interact with a multiplicity of molecular targets are likely to show greater efficacy in treating the core symptoms of schizophrenia.

 Dialogues Clin Neurosci. 2006;8:303-309.

Keywords: *human genome project; receptorome; druggable target; receptor*

Author affiliations: Departments of Biochemistry, Psychiatry, Neurosciences, and Oncology, National Institute of Mental Health Psychoactive Drug Screening Program, Case Western Reserve University Medical School, Cleveland, Ohio, USA (Current affiliation: Departments of Pharmacology, Medicinal Chemistry and Natural Products, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA)

Address for correspondence: Prof Bryan Roth, Dept of Pharmacology,University of North Carolina CB# 73658032 Burnett-Womack Bldg Chapel Hill, NC 27599, USA (e-mail: bryan_roth@med.unc.edu)

Psychiatric diseases represent a major cause of disability among individuals during their peak years of productivity (ages 15 to 44) and remain major causes of mortality in the developed world.[1] Because of this, governments and pharmaceutical companies have expended many billions of dollars on understanding the underlying causes of mental illnesses, and on discovering new and more effective treatments for them (Roth and Conn, unpublished report). The budget for the National Institute of Mental Health (NIMH)—the major funding agency for mental health-related research in the US—for the financial year 2006 stood at $1.4 billion, as stated on their Web site.[2] Despite this heavy investment, no psychiatric medications with greater efficacy than drugs discovered 50 years ago have yet appeared.[3,4] Thus, for example, clozapine (which was synthesized nearly 50 years ago[4]) continues to be the "gold standard" for treating schizophrenia.[5,6]

The recent sequencing and continued annotation of the human genome[7] and the tentative identification of a large number of schizophrenia susceptibility genes[8] have raised the possibility that molecular biology and its associated technologies will lead to new and improved treatments for schizophrenia and related disorders.[9] The assumption underlying this hope is that "we should finally make rapid progress identifying some of the vulnerability genes and thus critical pathways for the pathophysiology of the major mental illnesses…"[1] The hypothesis is that if we can understand the pathophysiological basis of these diseases—based on their molecular neurobiological underpinning—we will be better able to develop curative therapeutics (or "cure therapeutics"[1]) for schizophrenia and related disorders. Although this is a highly attractive hypothesis, it is founded on a number of assumptions, some of which are falsifiable, others of which are not (at least with the available technology). In this review, this hypothesis and its underlying assumptions will be exam-

ined, and suggestions will be put forward as to how molecular biology can (and cannot) provide tests of this hypothesis, as well as possibilities for novel medications for curative therapeutics of schizophrenia and related disorders.

Schizophrenia as a molecular disease

Currently, at least three overlapping paradigms drive the drug discovery effort for schizophrenia. These include, firstly, the molecular-genetic hypotheses which hypothesize strong effects of schizophrenia susceptibility genes.[8] A corollary of the molecular-genetic hypothesis is the proposal that targeting drugs at these genes might yield novel and more effective treatments for schizophrenia.[1,10] Secondly, the neuronal network hypotheses propose strong effects of altered neuronal integration in schizophrenia. The corollary of this hypothesis predicts that drugs which fundamentally reset the tone of networks of neuronal interactions will prove efficacious in treating schizophrenia.[4,11] Thirdly, the signal transduction hypothesis proposes that basic alterations in receptor-mediated signal transduction (either at the receptor or post-receptor levels) induce schizophrenia-like pathology. It follows that ameliorating altered signaling via specific medications which target receptor/post-receptor molecules will prove efficacious in treating schizophrenia.[12-16]

These general hypotheses are highly interconnected and interdependent. Thus, one could suggest, for instance, that schizophrenia arises because of mutation in a specific susceptibility gene—α7 nicotinic receptors for instance.[17] This mutation results in diminished α7 expression[18] which, in turn, leads to altered neuronal connectivity and signal transduction.[17] These alterations in neuronal signaling and connectivity lead to some of the symptoms of schizophrenia. The corollary is the proposal that α7 agonists will improve schizophrenia symptoms[19]—a hypothesis that is now being tested.

The underlying assumption of these lines of reasoning is that if one can identify the critical node *(Figure 1)* in the pathogenesis of schizophrenia and alter its functioning, one will more effectively treat schizophrenia. The implicit assumption is that only one (or a small number) of molecular targets function as critical nodes in the pathogenesis of schizophrenia. The role of molecular biology in such an undertaking is relatively straightforward: (i) identify the "disease-inducing molecules" (genetic linkage studies, candidate gene approaches); (ii) express the molecule in a way suitable for high-throughput-screening of large chemical libraries to identify candidate ligands with appropriate pharmacology (agonist, antagonist, partial agonist, inverse agonist, allosteric modulator[20]); (iii) provide molecular-target based assays for profiling candidate ligands at a large variety of other druggable targets to verify that the final lead compounds are suitably selective (or suitably nonselective[3,21]); and (iv) provide molecular-target based assays for profiling candidate ligands against various molecular targets which can lead to serious side effects. These can include prolongation of the Q-T interval via blockade of HERG K^+-channels,[22] agonism of 5-HT_{2B} serotonin receptors which can lead to cardiovascular side effects,[23] carcinogenicity, genotoxicity, and alteration of cytochrome P450 isoforms leading to altered pharmacokinetics (see ref 24 for instance). In the case of antipsychotic medications, weight gain and adverse metabolic side effects (likely mediated in part via H_1-histamine and 5-HT_{2C}-serotonin receptor blockade[34]) and extrapyramidal side effects (due to D_2-dopamine receptor blockade) occur frequently. Indeed, much of preclinical drug discovery in both industry and academia is driven primarily via molecular target-based screening and profiling technologies. Despite our ability to screen millions of drug-like compounds at hundreds of druggable targets which comprise the "druggable genome,"[25,26] no novel molecularly targeted treatments for schizophrenia have been approved. Indeed, as already mentioned, clozapine continues to be the "gold-standard treatment" for schizophrenia.

The critical node assumption has not (yet) yielded better drugs for schizophrenia

Based on the "critical node" assumption, a large number of potential nodes have been identified for therapeutic drug discovery. These have been identified via the three general strategies outlined above (eg, molecular genetic, neuronal network, or signal transduction) and a large number of these candidate nodes have been a theme of research over the past decade. As we have recently summarized as part of a larger study of psychiatric drug discovery, nearly 150 investigational compounds directed against many individual molecular targets ("nodes") have been subjected to at least early-phase clinical trials (Roth and Conn, unpublished report). Representative compounds for each node are listed in *Table I*. In this table, antipsychotic drugs have been classified based on

molecular target (eg, "node")/targets ("nodes") and whether the compounds were validated with preclinical and clinical studies. Lastly, it is indicated whether the compounds were found, based on clinical trials, to be superior to a standard comparator medication (typically haloperidol). Based on the currently available data, we were unable to find any evidence to support the hypothesis that targeting any single molecular target ("node") other than D_2 dopamine receptors will yield a drug which effectively treats the core symptoms of schizophrenia. Additionally, we were unable to find any support for the hypothesis that drugs targeting a single node are more effective at treating schizophrenia than drugs targeting a large number of nodes. Indeed, clozapine, which targets at least 50 nodes, remains superior to all other medications.[3,5] The results obtained are consistent with the proposal that "D_2 dopamine receptors represent the critical node in schizophrenia pathogenesis."[13] It is unknown

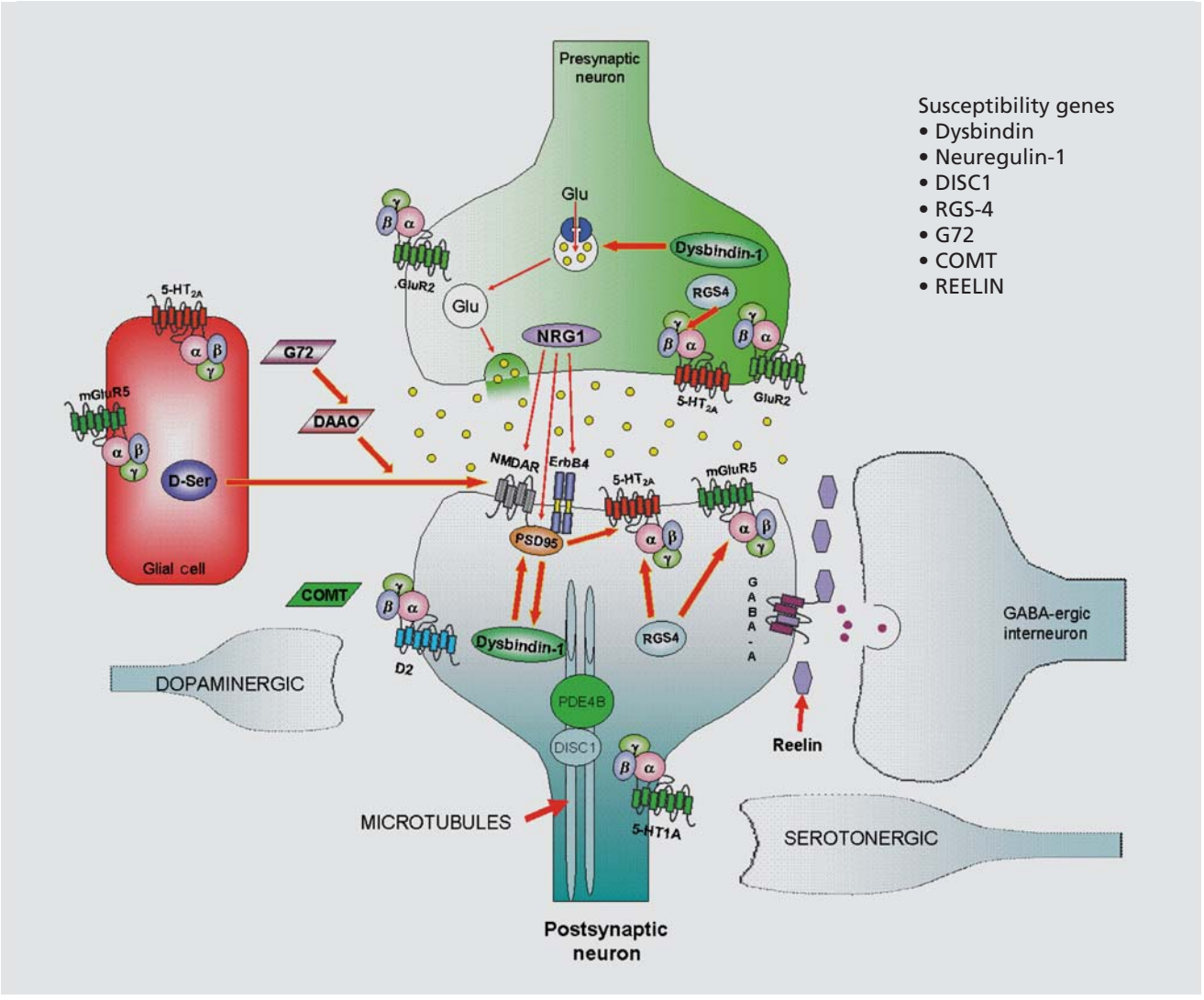


Figure 1. Schizophrenia susceptibility genes are localized in overlapping neuronal pathways. Shown in diagrammatic form are the presumed localizations of various schizophrenia susceptibility gene products in a model synapse in the prefrontal cortex. As shown, a typical pyramidal neuron fiber receives inputs from dopaminergic, serotonergic, glutamatergic, and GABA-ergic neurons. The various susceptibility genes indicated may modulate pre- or postsynaptic glutamatergic functioning. Antipsychotic drugs mainly affect biogenic amine receptor activities which may be either pre- or postsynaptic in nature. GABA, γ-aminobutyric acid

whether any single molecular target of greater promise will ever be found.

There are many ways in which these findings can be interpreted, although each interpretation relies mainly on untested assertions. A typical criticism one can make of these findings is that "we have not yet found the *critical node*" and that once this key node is discovered, the pathway towards drugs with greater efficacy and fewer side effects will be clarified. The untested assumptions are (i) that such a special node associated with efficacy exists; (ii) that it can be discovered; and (iii) that, once discovered, using techniques of molecular biology, a drug can be designed to target it. An implicit assumption underlying this argument relates to the need for an enhanced understanding of the molecular pathogenesis of schizophrenia in order to discover and validate suitable molecular targets.[1,9]

Based upon our current understanding of the molecular pathogenesis of schizophrenia, *no critical node other than the D_2 dopamine receptor has yet been convincingly and reproducibly elucidated*, although a large number of candidate genes and susceptibility factors have been

Node (molecular target)	Representative drug	Preclinical evidence of efficacy*	Results from randomized clinical trials	Efficacy > haloperidol	Side effects
D_2 dopamine antagonist	Haloperidol, amisulpride	Many	Effective	Equivalent	EPS
D_2 dopamine partial agonist	Aripiprazole	Many	Effective	Equivalent	Activation
Highly promiscuous antagonist (40+ nodes)	Clozapine	Many	Effective	More effective	Agranulocytosis, weight gain, sedation, seizures
Moderately promiscuous antagonist (20+ nodes)	Olanzapine	Many	Effective	Equivalent	Weight gain, sedation
Mildly promiscuous antagonist (10-20 nodes)	Risperidone	Many	Effective	Equivalent	Weight, gain, sedation, ? EPS with higher doses
Promiscuous agonist (40+nodes;partial agonist at >3)	N-desemethyl-clozapine	Many	Unknown	Unknown	Unknown
5-HT_{2A} antagonist	SR46349B	Many	Possibly effective	Possibly equivalent	Minimal
NK-3 antagonist	SR142801	Partial	Possibly effective (clinical development ceased)	Equivalent	Minimal
D_4 antagonist	Belaperidone	Partial	No	No	Worsening of psychosis?
D_3 antagonist	LU-201640	Partial	Ongoing**	Ongoing	Ongoing
D_1 antagonist	BSF-78438	Partial	Dropped***	Dropped	Dropped
Sigma-1 antagonist	BMY 14802	Partial	Ineffective	Ineffective	Perhaps worsening of psychosis
AMPA 1 glutamate modulator	Org-24448	Partial	Ongoing	Ongoing	Ongoing
$mGluR_2$ glutamate agonist	LY-341495	Partial	Ongoing	Ongoing	Ongoing
CB-1 cannibinoid antagonist	SR141716	Partial	Ineffective	Ineffective	Dropped
NT-1 neurotensin antagonist	SR48692	Partial	Ineffective	Ineffective	Dropped
α7-Nicotinic agonist/partial agonist	MEM-3454	Partial	Ongoing	Ongoing	Ongoing
NMDA glutamate modulator	D-serine	Partial	Perhaps partially effective	Ongoing	Ongoing
PDE10A antagonist	Papaverine	Partial	Unknown	Unknown	Unknown
α_2-Adrenergic agonist	Clonidine	Partial	Perhaps partial as augmentation	Unknown	Unknown

Table I. Multiple candidate nodes have been subjected to testing as targets for treating schizophrenia. This shows an abstracted analysis from a recent study[2] examining the evidence for and against various molecular-target based approaches for treating schizophrenia. *, various animal models which have been tested and for which the drug has efficacy; **, clinical trials are ongoing and information is not available; ***, dropped from development with no further data available; EPS, extrapyramidal syndrome.

described. These include neuregulin-1,[27] dysbindin,[28] disrupted in schizophrenia-1 (*DISC-1*)[29] and many others (eg, reelin, regulator of G protein signaling-4, catechol-O-methyltransferase, mGluR3 glutamate receptor, and so on; see ref 8 for recent review). As we[3] and others[30] have pointed out *(Figure 1)* these susceptibility gene products are found in a variety of cell types (both neuronal and glial) and show differential subcellular localizations. As *Figure 1* shows, the molecular targets identified are frequently found in circuits which are targeted by drugs with a "promiscuous" pharmacology (eg, clozapine). No single node is an obvious target for therapeutic drug discovery efforts, although nearly all of the identified nodes have been reported to be targets of therapeutic drug discovery (Roth and Conn, unpublished report).

Another possibility is that schizophrenia can be most effectively treated by influencing several nodes simultaneously.[3] Indeed, based on the demonstrated superiority of clozapine for treatment-resistant schizophrenia[5] and the relative inferiority of all other medications,[6] there is strong support for this hypothesis. A great deal of effort has been expended to discover an optimal clozapine-mimetic devoid of the side effects of clozapine which include agranulocytosis, seizures, sialorrhea, weight gain, sedation, and hypotension. We, and others, have suggested that the massively parallel screening of large numbers of molecular targets allows one to efficiently discover "toxic" vs "therapeutic" targets.[32-34] Antipsychotic drug-induced weight gain might be due to H_1-histamine and 5-HT_{2C}-receptor blockade,[35,36] agranulocytosis to H_4 histamine agonism,[2] sedation to H_1 histamine antagonism,[4] and so on. Thus far, these molecular targets implicated in clozapine's side effects (H_1-histamine, H_4-histamine, 5-HT_{2C} serotonin) are not identical with those targets thought to be involved in its superiority as an antipsychotic drug (5-HT_{2A} serotonin, D_4-dopamine, 5-HT_6 and 5-HT_7 serotonin). A problem with the approach of designing selectively nonselective drugs is that it is very difficult to rationally design in new pharmacological properties during the drug discovery process.[24] This is an emerging paradigm, however, and some successful strategies have recently been elucidated.[37]

A systems level approach

The neuronal systems approach similarly proposes that there might be crucial nodes in the network that are amenable to target-based discovery efforts.[4] Spedding and colleagues have cogently argued that a systems-level approach using animal models will lead to more effective treatment for psychiatric diseases.[4] Based on a model which involves specific alterations in hippocampal-cortical circuitry, they propose testing compounds in animals in which these circuits are disrupted by phenycyclidine (PCP). In support of this systems-level approach, nearly every approved antipsychotic drug will ameliorate PCP-induced alterations in neuronal functioning.[37] However, it is also true that drug classes with demonstrated ability to ameliorate PCP-induced deficits (eg, 5-HT_{2A} antagonists[38]) are only marginally effective in treating schizophrenia.[39,40] Thus, in vivo systems-level screens can be highly effective tools to verify in vivo actions of putative atypical antipsychotic drugs. It does not appear that any of the available in vivo screening models are able to predict relative efficacy at treating schizophrenia, however. In addition, none of the available models appears to adequately recapitulate the entirety of the human phenotype.[37]

One can easily provide the counterargument that a "suitable animal model will eventually be found which recapitulates the schizophrenia phenotype," although it is also plausible that "no suitable preclinical model will ever be found which adequately recapitulates schizophrenia pathology." Clearly, despite decades of research we have not yet discovered an adequate preclinical model, and it is within the realm of possibility that "schizophrenia is a uniquely human disease which cannot be adequately modeled in rodents." In large measure, this is likely to be due to the fact that a number of genetic "hits" as well as nongenomic factors converge to produce the final phenotype in humans.[41] At present, we have no way to predict either way, and continued research in this arena will be based more on untested assumptions than on data.

Is schizophrenia similar to hypertension in being complex, polygenic, and epigenetic?

Another possibility is that schizophrenia represents a complex disease with genetic and epigenetic factors and which is both chronic and progressive, resulting in irreversible end-organ damage—similar to hypertension. Indeed, there is accumulating evidence for epigenetic factors involved in the etiology of schizophrenia—particularly relating to reelin.[42-45] There has also been abundant evidence accumulated over the past several decades that schizophrenia is associated with subtle but reproducibly

documented neurodegeneration (reviewed in refs 46, 47). Accordingly, optimal treatment of schizophrenia would be similar to that for other progressive and complex diseases such as hypertension, where individuals at risk would be identified and then treated to avoid end-organ damage. Such an approach has already been attempted, with a mixed degree of success.[48] In this study, individuals at risk were identified and then prophylactically treated with placebo or olanzapine. Although the results were not statistically significant, there was a trend toward protection of conversion to overt psychosis among individuals treated with olanzapine.[48]

Conclusion

As is clear from the foregoing, the tools of molecular biology can, at least theoretically, accelerate drug discovery in schizophrenia. In the main, molecular biological approaches have been more useful in providing reagents for high-throughput screening campaigns than for providing better animal models—at least to date. With the continued discovery of schizophrenia susceptibility genes, it is at least conceivable that better preclinical models will be produced. To a great degree, lack of progress in developing more effective antipsychotic drugs has stemmed mainly from the failure both to fully appreciate the pharmacological robustness of clozapine and to discover medications which reproduce the essential features without producing serious side effects. It is not clear whether any of the paradigms outlined will lead to more effective medications, although it is likely that continued molecular target-based screening will eventually yield medications with fewer side effects. ❑

The work from the author's lab was supported entirely by grants from the National Institute of Health (MH57635, MH61887, DA017237) and the NIMH Psychoactive Drug Screening Program.

REFERENCES

1. Insel TR, Scolnick EM. Cure therapeutics and strategic prevention: raising the bar for mental health research. *Mol Psychiatry*. 2006;11:11-17.
2. National Institutes of Mental Health. Facts about NIMH. Available at: http://www.nimh.nih.gov/about/nimh.cfm. Accessed August 8, 2006.
3. Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov*. 2004;3:353-359.
4. Spedding M, Jay T, Costa e Silva J, Perret L. A pathophysiological paradigm for the therapy of psychiatric disease. *Nat Rev Drug Discov*. 2005;4:467-476.
5. Kane J, Honigfield G, Singer J, Meltzer HY, Group at CCS. Clozapine for the treatment-resistant schizophrenic. *Arch Gen Psychiatry*. 1988;45:789-796.
6. McEvoy JP, Lieberman JA, Stroup TS, Davis SM, Meltzer HY, Rosenheck RA, et al. Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *Am J Psychiatry*. 2006;163:600-610.
7. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. *Science*. 2001;291:1304-1351.
8. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry*. 2005;10:40-68.
9. Insel TR, Collins FS. Psychiatry in the genomics era. *Am J Psychiatry*. 2003;160:616-620.
10. Sawa A, Snyder SH. Schizophrenia: neural mechanisms for novel therapies. *Mol Med*. 2003;9:3-9.
11. Hyman SE, Nestler EJ. Initiation and adaptation: a paradigm for understanding psychotropic drug action. *Am J Psychiatry*. 1996;153:151-162.
12. Carlsson M, Carlsson A. Systems within the basal ganglia: implications for schizophrenia and Parkinson's disease. *Trends Neurosci*. 1990;13:272-276.
13. Carlsson A. The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology*. 1988;1:179-186.
14. Meltzer HY. Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology*. 1989;99:S18-S27.
15. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991;148:1301-1308.
16. Freedman R, Adler LE, Bickford P, et al. Schizophrenia and nicotinic receptors. *Harv Rev Psychiatry*. 1994;2:179-192.
17. Freedman R, Coon H, Myles-Worsley M, et al. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A*. 1997;94:587-592.
18. Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol Psychiatry*. 1995;38:22-33.
19. Martin LF, Kem WR, Freedman R. Alpha-7 nicotinic receptor agonists: potential new candidates for the treatment of schizophrenia. *Psychopharmacology (Berl)*. 2004;174:54-64.
20. Neubig RR, Spedding M, Kenakin T, Christopoulos A. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. *Pharmacol Rev*. 2003;55:597-606.
21. Morphy R, Kay C, Rankovic Z. From magic bullets to designed multiple ligands. *Drug Discov Today*. 2004;9:641-651.
22. Recanatini M, Poluzzi E, Masetti M, Cavalli A, De Ponti F. QT prolongation through hERG K(+) channel blockade: current knowledge and strategies for the early prediction during drug development. *Med Res Rev*. 2004;25:133-166.
23. Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, et al. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*. 2000;102:2836-2841.
24. Lessard E, Yessine MA, Hamelin BA, O'Hara G, LeBlanc J, Turgeon J. Influence of CYP2D6 activity on the disposition and cardiovascular toxicity of the antidepressant agent venlafaxine in humans. *Pharmacogenetics*. 1999;9:435-443.

Contribuciones de la biología molecular al descubrimiento de fármacos antipsicóticos: ¿promesas cumplidas o incumplidas?

Esta revisión resume los diversos paradigmas conceptuales que existen para el tratamiento de la esquizofrenia e indica cómo la biología molecular y las tecnologías para el descubrimiento de fármacos pueden acelerar el desarrollo de nuevos medicamentos. Aun no se dispone de información convincente acerca de la existencia de una molécula específica, que pueda transformarse en un medicamento, y que de encontrarse pueda dar origen a fármacos más eficaces que cualquiera de los actualmente disponibles. Se sugiere, en cambio, que es probable que fármacos que interactúan con una multiplicidad de blancos moleculares muestren mayor eficacia en el tratamiento de los síntomas centrales de la esquizofrenia.

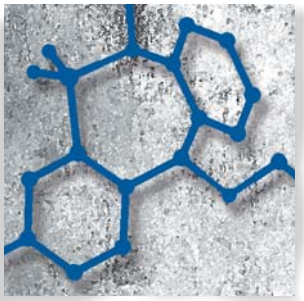
Apports de la biologie moléculaire à la découverte des médicaments antipsychotiques : promesses tenues ou non ?

Cet article résume les différents modèles conceptuels de traitement de la schizophrénie et montre comment la biologie moléculaire et les technologies de découverte des médicaments peuvent accélérer le développement de nouveaux traitements. Nous ne disposons pas encore de données convaincantes sur l'existence d'une molécule décisive, transformable en médicament qui, si elle était choisie, déboucherait sur des traitements plus efficaces que ceux disponibles actuellement. Il est plutôt suggéré que les médicaments interagissant avec les nombreuses cibles moléculaires seraient plus efficaces dans le traitement des symptômes clés de la schizophrénie.

25. Hopkins AL, Groom CR. The druggable genome. *Nat Rev Drug Discov*. 2002;1:727-730.
26. Russ AP, Lampel S. The druggable genome: an update. *Drug Discov Today*. 2005;10:1607-1610.
27. Stefansson H, Sigurdsson E, Steinthorsdottir V, et al. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet*. 2002;71:877-892.
28. Straub RE, Jiang Y, MacLean CJ, et al. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am J Hum Genet*. 2002;71:337-348.
29. St Clair D, Blackwood D, Muir W, et al. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet*. 1990;336:13-16.
30. Harrison PJ, Owen MJ. Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet*. 2003;361:417-419.
31. Vortherms TA, Roth BL. Receptorome screening for CNS drug discovery. *Drugs*. 2005;8:491-496.
32. Armbruster BN, Roth BL. Mining the receptorome. *J Biol Chem*. 2005;280:5129-5132.
33. Fliri AF, Loging WT, Thadeio PF, Volkmann RA. Biological spectra analysis: Linking biological activity profiles to molecular structure. *Proc Natl Acad Sci U S A.* 2005;102:261-266.
34. Kroeze WK, Hufeisen SJ, Popadak BA, et al. H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology*. 2003;28:519-526.
35. Wirshing DA, Wirshing WC, Kysar L, et al. Novel antipsychotics: comparison of weight gain liabilities. *J Clin Psychiatry*. 1999;60:358-63.
36. Morphy R, Rankovic Z. Designed multiple ligands. An emerging drug discovery paradigm. *J Med Chem*. 2005;48:6523-6543.
37. Geyer MA, Ellenbroek B. Animal behavior models of the mechanisms underlying antipsychotic atypicality. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003;27:1071-1079.
38. Varty GB, Bakshi VP, Geyer MA. M100907, a serotonin 5-HT2A receptor antagonist and putative antipsychotic, blocks dizocilpine-induced prepulse inhibition deficits in Sprague-Dawley and Wistar rats. *Neuropsychopharmacology*. 1999;20:311-321.
39. Potkin SG, Shipley J, Bera R, et al. Clinical and PET Effects of M100907, a selective 5HT-2A receptor antagonist. *Schizophr Res*. 2001;49:242.
40. Meltzer HY, Arvanitis L, Bauer D, Rein W. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *Am J Psychiatry*. 2004;161:975-984.
41. Ellenbroek BA. Animal models in the genomic era: possibilities and limitations with special emphasis on schizophrenia. *Behav Pharmacol*. 2003;14:409-417.
42. Costa E, Chen Y, Davis J, et al. REELIN and schizophrenia: a disease at the interface of the genome and the epigenome. *Mol Interv*. 2002;2:47-57.
43. Tremolizzo L, Carboni G, Ruzicka WB, et al. An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability. *Proc Natl Acad Sci U S A*. 2002;99:17095-17100.
44. Abdolmaleky HM, Cheng KH, Russo A, et al. Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. *Am J Med Genet B Neuropsychiatr Genet*. 2005;134:60-66.
45. Grayson DR, Jia X, Chen Y, et al. Reelin promoter hypermethylation in schizophrenia. *Proc Natl Acad Sci U S A*. 2005;102:9341-9346.
46. Berger GE, Wood S, McGorry PD. Incipient neurovulnerability and neuroprotection in early psychosis. *Psychopharmacol Bull*. 2003;37:79-101.
47. de Haan L, Bakker JM. Overview of neuropathological theories of schizophrenia: from degeneration to progressive developmental disorder. *Psychopathology*. 2004;37:1-7.
48. McGlashan TH, Zipursky RB, Perkins D, et al. Randomized, double-blind trial of olanzapine versus placebo in patients prodromally symptomatic for psychosis. *Am J Psychiatry*. 2006;163:790-799.

Membrane transporter proteins: a challenge for CNS drug development

François Girardin, MD



The consequences of transporters on central nervous system (CNS) drug development are becoming increasingly important, due to their influence on clinical outcome. Membrane transporters provide insight into the mechanisms of treatment failure, adverse drug reactions, and individual differences in the management of neurological and psychiatric disorders.

The presence of uptake and efflux transporters in capillary endothelial cells mediates drug transport from the

Drug transporters are membrane proteins present in various tissues such as the lymphocytes, intestine, liver, kidney, testis, placenta, and central nervous system. These transporters play a significant role in drug absorption and distribution to organic systems, particularly if the organs are protected by blood-organ barriers, such as the blood-brain barrier or the maternal-fetal barrier. In contrast to neurotransmitters and receptor-coupled transporters or other modes of interneuronal transmission, drug transporters are not directly involved in specific neuronal functions, but provide global protection to the central nervous system. The lack of capillary fenestration, the low pinocytic activity, and the tight junctions between brain capillary and choroid plexus endothelial cells represent further gatekeepers limiting the entrance of endogenous and exogenous compounds into the central nervous system. Drug transport is a result of the concerted action of efflux and influx pumps (transporters) located both in the basolateral and apical membranes of brain capillary and choroid plexus endothelial cells. By regulating efflux and influx of endogenous or exogenous substances, the blood-brain barrier and, to a lesser extent, the blood-cerebrospinal barrier in the ventricles, represents the main interface between the central nervous system and the blood, ie, the rest of the body. As drug distribution to organs is dependent on the affinity of a substrate for a specific transport system, membrane transporter proteins are increasingly recognized as a key determinant of drug disposition. Many drug transporters are members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter superfamily or the solute-linked carrier (SLC) class. The multidrug resistance protein MDR1 (ABCB1), also called P-glycoprotein, the multidrug resistance-associated proteins MRP1 (ABCC1) and MRP2 (ABCC2), and the breast cancer-resistance protein BCRP (ABCG2) are ATP-dependent efflux transporters expressed in the blood-brain barrier. They belong to the superfamily of ABC transporters, which export drugs from the intracellular to the extracellular milieu. Members of the SLC class of solute carriers include, for example, organic ion transporting peptides, organic cation transporters, and organic ion transporters. They are ATP-independent polypeptides principally expressed at the basolateral membrane of brain capillary and choroid plexus endothelial cells that also mediate drug transport through central nervous system barriers.

Dialogues Clin Neurosci. 2006;8:311-321.

Keywords: *drug transporter; blood-brain barrier; drug distribution; ATP-binding cassette transporter; solute-linked carrier*

Author affiliations: Unit of Clinical Psychopharmacology, Geneva University Hospitals, Chênes-Bourg, Geneva, Switzerland

Address for correspondence: Dr François Girardin, Unit of Clinical Psychopharmacology and Department of Community Medicine, Geneva University Hospitals, CH-1225 Chênes-Bourg, Geneva, Switzerland
(e-mail: françois.girardin@hcuge.ch)

Selected abbreviations and acronyms

ABC	*adenosine triphosphate-binding cassette*
ATP	*adenosine triphosphate*
BBB	*blood-brain barrier*
BCRP	*breast cancer resistance protein*
CNS	*central nervous system*
CSB	*(blood) cerebrospinal barrier*
MDR	*multidrug resistance*
MRP	*multidrug resistance-associated protein*
OAT	*organic ion transporter*
OATP	*organic anion transporting peptide*
OCT	*organic cation transporter*
SLC	*solute-linked carriers*

bloodstream to the organs. For example, these membrane transporters play an essential role in the digestive tract, the kidney, and the liver, as well as other organic systems such as the blood cells, the placenta, and the central nervous system.[1]

All cells selectively transport endogenous and exogenous compounds across their membrane to maintain an intracellular milieu distinct from the outer one; this is achieved in part by the membrane transporters, which are multispecific transport proteins. These transporters display physiologic functions in terms of transporting endogenous compounds, eg, hormones, amino acids, bile acids, and lipids.[2-4] Moreover, membrane transporter mutations may lead to severe genetic disorders such as cystic fibrosis (ABCC7),[5] immune deficiency (ABCB2 and ABCB3),[6] intrahepatic cholestasis of pregnancy (ABCB11),[7] persistent hypoglycemia of infancy (ABCC8),[8] X-linked adrenoleukodystrophy (ABCD1),[9] X-linked ataxia with sideroblastic anemia (ABCB7),[10] and retinal degeneration (ABCA4).[11,12]

This review focuses on the functional significance of membrane transporters as drug carriers: their role is constantly increasing in current medical practice, as they represent a key factor in clinical outcome.[7,13,14]

Physiological aspects

The blood-brain barrier (BBB) is a physical barrier, and maintains a given extracellular environment for neurons and glial cells in the CNS. The BBB is formed by the connection of closely sealed tight junctions between the capillary endothelial cells, which are not fenestrated and which display minimal pinocytosis *(Figure 1)*. The capillary endothelial cells form a polarized barrier similar to that located in the retina or in the renal proximal tubule, which regulates diffusion of molecules across the BBB, and limits the entry of xenobiotics via paracellular pathways by intercellular tight junctions.[15-17] Not all cerebral blood vessels are closely sealed with tight junctions: fenestrated capillaries are found in the pituitary gland and

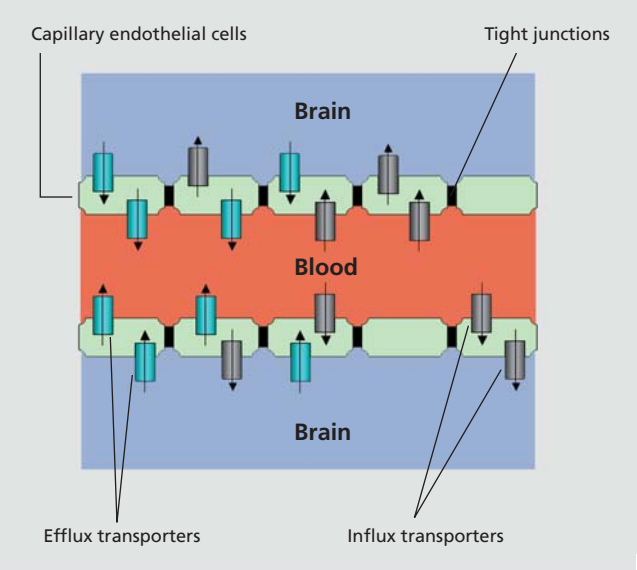


Figure 1. The blood-brain barrier and drug transporters in the capillary endothelial cells.

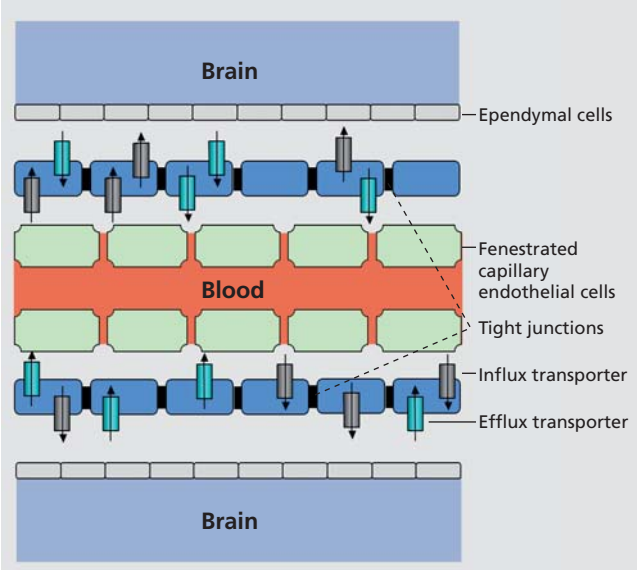


Figure 2. The cerebrospinal barrier and drug transporters in the choroid plexus cells.

in the cirumventricular organs such as the area postrema, the lamina terminalis, and the median eminence.[18]

The choroid plexus is a highly vascularized epithelial organ which secretes the cerebrospinal fluid and regulates its composition through active and selective transport processes; it has an active role in the cleansing of the cerebrospinal fluid of endogenous and exogenous compounds.[19] The blood cerebrospinal barrier (CSB) is considered as the second fluid barrier protecting the central nervous system: it is principally formed by epithelial cells of the choroid plexus in the ventricles and the arachnoid membrane. Like the brain capillary endothelial cells, the choroid plexus epithelial cells are connected by high-resistance tight junctions, which closely separate the blood from the cerebrospinal fluid compartment *(Figure 2)*.[20]

Together with the BBB and the CSB, the membrane transporter systems represent further gatekeepers to the CNS; these play a critical role in drug disposition.[21] Membrane transporters either enhance or restrict drug distribution to the target organs. Depending on their main function, these membrane transporters are divided into two categories: the efflux (export) and the influx (uptake) transporters. Influx transport proteins facilitate and efflux transporters limit drug passage through membrane barriers such as the BBB or the CSB.[22] Several membrane transporters are found at the apical and basolateral epithelial cell membrane of the brain capillary and of the choroid plexus endothelial cells *(Figure 3)*.[23]

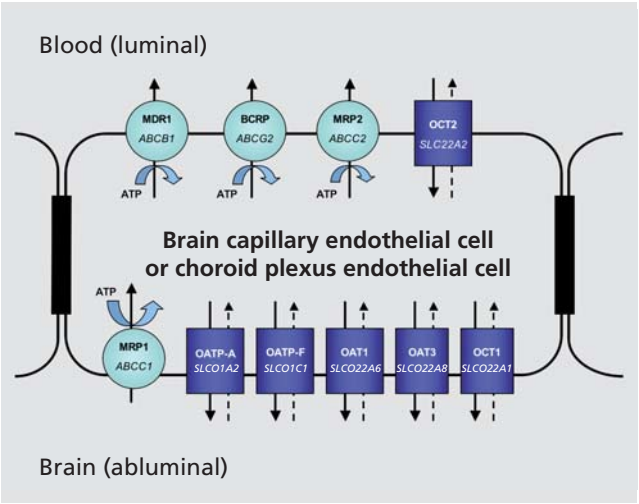


Figure 3. Examples of drug transporters and localization in cells forming the CNS barriers. CNS, central nervous system; SLC, solute-linked carrier.

Pharmacological aspects

Drug absorption from the systemic circulation into the CNS was previously considered a passive process that depended on drug physicochemical properties such as molecular size, lipophilicity, and the pK_a of a drug. Although the physicochemical properties of a medication do affect its absorption and access to target organs, transporter proteins have a major role in the overall drug distribution process through their targeted expression in tissue such as the brain. Until recently, most pharmacokinetic studies focused only on the role of drug-metabolizing enzymes as a key determinants of drug disposition.[24]

Phase I enzymatic reactions are mainly represented by the cytochrome P450 mono-oxygenase system, as well as by other enzymes such as pseudocholinesterase or alcohol dehydrogenase. Phase I enzymatic reactions modify the chemical structure of the compound itself with either loss of pharmacological activity or, on the contrary, when prodrugs are administered, enhanced biological activity through biotransformation into an active metabolite.

In contrast, Phase II enzymatic reactions are conjugation reactions that lead to the formation of a covalent linkage with an endogenously derived compound such as glucuronic acid, sulfate, glutathione, or amino acids into highly polar conjugates excreted into the bile or urine: N-acetyltransferase 2 or catechol-O-methyltransferase are examples of enzymes that catalyze phase II reactions. Drug membrane transporters enable access of compounds to phase I reactions and further elimination after phase II reactions.

Thus, drug uptake transporters deliver the drug to an intracellular enzymatic detoxification system, whereas drug efflux transporters decrease the intracellular load from the detoxification system. Inhibition of transmembrane transporters may lead to a lower substrate uptake with a poorer intracellular access of the drug to the enzymatic systems.[25]

This synergy between metabolizing enzymes and drug transporters accounts for the distribution to brain tissues, and determines the pharmacokinetic profile of CNS drugs.[26] Furthermore, substrate similarity is well documented between membrane transporters and cytochrome P450 enzymes. For instance, substrates of the PGP multidrug resistance protein (MDR1) are usually substrates of the CYP3A4, digoxin and fexofenadine excepted.[27]

Characterization of drug transporters

Many drug transporters are members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter superfamily or the solute-linked carrier (SLC) class. The ABC superfamily (ABC transporters) comprises the main efflux membrane transporters for drug elimination, and the solute-linked carriers (SLC transporters) are invlolved with influx and efflux transport function for drug uptake and export.[28]

ABC transporters

The ABC transporters are transmembranous proteins found in all animal species; they require energy from ATP hydrolysis to actively remove compounds from the cell, often against a high concentration gradient. They are composed of two transmembrane domains that form a pathway through which the substrates move, and by two nucleotide-binding domains located at the cytoplasmatic face of the membrane, providing ATP hydrolysis to allow substrate translocation across cellular membranes.[29] Members of the ABC superfamily are classified as such according to consensus sequences including both domains (Walkers A and Walkers B ATP-binding motifs), as well as the ABC signature (C motif).[30] To date, 49 human ABC family members have been identified, divided into seven different subfamilies.

The ABC transporter superfamily includes medically important members such as the multidrug resistance-associated protein 1 (MRP1) located at the basolateral plasma membrane domain (abluminal), the multidrug resistance-associated protein 2 (MRP2), the breast cancer resistance protein (BCRP), and MDR1 (also known as PGP) localized at the apical membrane (luminal). MDR1 (encoded ABCB1), BCRP (encoded ABCG2), MRP1 (encoded ABCC1), and MRP2 (encoded ABCC2) were identified in the BBB at the luminal side of the capillary endothelial cells, MRP1 excepted *(Figure 3)*.

The substrates handled by the ABC transporters include a wide range of endogenous and exogenous compounds and diverse type of molecules, from organic cations and anions to larger molecules such as large polypeptides or therapeutic agents. For instance, MRP1 has preferential transport of anionic compounds such as sulfate conjugates or glutathione, whereas MDR1 shows broader substrate specificity.[31,32]

Variation of ABC transporter activity by drug-drug interactions, genetic polymorphisms, and overexpression is considered as a major cause of treatment failure, interindividual variability, and adverse drug reactions.[25,33] However, randomized controlled studies on these issues with antidepressants, antipsychotics, or mood stabilizers in humans are still lacking.

ABC transporters are considered as the most relevant determinants of efflux transport and provide multiple barriers in the brain capillary and choroid plexus endothelial cells.[34] A multidrug resistance feature is associated with a poorer clinical outcome in several CNS disorders.[24] Furthermore, several ABC transporters were directly implicated in drug delivery to brain neoplasms and in the response to therapeutic agents.[35] For instance, the expression of the ABC transporters ABCC4 and ABCC5 was associated with an astrocytic phenotype with higher chemoresistance of astrocytic tumors compared with oligodendrogliomas.[36] Recently, the ABC transporters were also found to be associated with pharmacoresistance to anticonvulsant drugs in patients with intractable mesial temporal lobe epilepsy.[37]

MDR1 activity significantly decreases during aging with, consecutively, an increased brain exposure to drugs and toxins in elderly subjects.[38] Furthermore, impaired MDR1 function is reported as a predisposing factor in the development of neurodegenerative diseases such as Parkinson's disease or sporadic Alzheimer's dementia.[39] Pathologic accumulation of amyloid β in Alzheimer's disease may result from an impaired MDR1 activity, as amyloid β is considered as a substrate for MDR1.[13]

SLC transporters

The SLC class of solute carriers consists of specific membrane transporters that mediate sodium-independent transmembrane solute transport: it is divided into 43 human families based upon amino acid homology of at least 25% between family members. To date, nearly 300 genes have been identified.[40] The Human Genome Organization (HUGO) Nomenclature Committee Database provides information about new transporter families of the SLC gene series (SLC transporters-gene nomenclature, SLCO).[41] Members of the SCLO superfamily are not only expressed in the BBB and in the choroid plexus, but also in the small intestine, the liver, the kidney, the blood-testis barrier, and the placenta.[42,43]

The SLCO superfamily of solute carriers includes carriers,

such as the organic anion transporting protein family (OATPs), the organic anion transporter family (OATs, *SLCO22A*), and the organic cation transporter family (OCTs, *SLCO22A*). The organic cation/carnitine transporter (OCTN, *SLCO22A*), the monocarboxylate transporter family (MCTs, *SLCO16*), and the peptide transporter (PEPT, *SLCO15A*) may represent further important SLC families, and their function as CNS barriers is currently under investigation.[44,45] For example, α-hydroxybutyrate (GHB), a therapeutic agent for catalepsy with narcolepsy, undergoes passive diffusion through the BBB but also the MCT1 carrier-mediated process, that is saturable and can be inhibited.[46] Proof-of-concept studies are being conducted to provide better insights into GHB therapy and GHB toxicity by means of transport inhibitors.[47]

Several in vitro and in vivo data indicate that OATP1A2, OATP1C1, and OATP2B1 (members of the SLCO21A family), and OAT1, OAT2, OCT1, OCT2, and OCT3 (members of the SLCO22A family) are expressed in the murine and human brain, and mediate drug transport through the CNS barriers.[2-4,36,41,48,49]

The SLCO21A family is referred to as the OATP family: these transporters consist of 12 transmembrane domain proteins, whose substrates are anionic amphipathic high-molecular-weight molecules that bind to albumin.[40] The transport mechanism is based upon anion exchange coupled to cellular uptake of organic compounds with the efflux of bicarbonate, glutathione, and conjugates.

The SLC22A transporters include OCTs including OCTN, and OATs that also consist of 12 transmembrane domain proteins, but with different substrate specificity. Indeed, OATPs not only mediate uptake of anionic, but also neutral and cationic, compounds. OCT members are mainly unidirectional porters, whereas OAT members act as anion exchangers. The organic anion transporting proteins (OATPs), OATs, and the OCTs represent the major uptake transport systems that mediate organic compound transport activities at the apical and the basolateral plasma membrane domains.

Drug transporter polymorphisms

The expression of transport proteins localized in the membranes of various organs are significant determinants of the pharmacokinetics of therapeutic agent including at the level of the CSB and the BBB.[33,50-53]

There is genetic polymorphism of drug transporters in the structure of genes and in the number of alleles. The *MDR1* gene has been particularly well investigated and several *MDR1* polymorphisms have been found: many of them determine membrane transporters expression in the BBB and in the CNS with variable drug transport activity.[26,54,55] Such medically relevant polymorphisms are called nonsynonymous polymorphisms, as they directly condition the drug transporter function with potentially variable clinical outcomes.

The functional significance of different *MDR1* expression for drug disposition was mainly studied with *MDR1* knockout mice. For instance, *MDR1* knockout mice are 50- to 100-fold more sensitive to the neurotoxic pesticide ivermectine, and the accumulation of this drug in the CNS was 80- to 100-fold greater when compared with control mice.[56]

In humans, several mutations resulting in several *MDR1* single nucleotide polymorphisms (SNPs) have been identified, but only those at positions 2677 and 3435 seem to be associated with changes in PGP expression and function.[25,57,58] In contrast to the P450 drug-metabolizing enzymes such as CYP2C9, CYP2C19, and CYP2D6, for which loss of function mutations or gene amplification manifests as distinct phenotypes in the population (eg, poor, intermediate, extensive, or ultrarapid metabolizers), the impact of *MDR1* polymorphisms on pharmacokinetics is moderate: no definite *MDR1* phenotype is recognized in humans.[59] There is no complete loss of transport function when polymorphisms are present: the genotype-related differences in the *MDR1* expression between, eg, the 3435 genotype, remains moderate with substantial overlap.[59] However, the difference between clinical outcomes may be in some conditions very impressive: patients with drug-resistant epilepsy were much more likely to have the CC genotype at ABCB1 3435 than the TT genotype (odds ratio: 2.66).[60] Furthermore, ABC transporter polymorphisms are not only associated with resistance to treatment or failure, for example, for anticonvulsants, cytostatics, or antibiotics, but they also determine the incidence of adverse drug events.[50,53,57,60-63] Some examples of clinical effects and potential implications associated with human drug transporter polymorphisms are listed in *Table I*.

Interestingly, the clinical impact of single nucleotide polymorphisms on genetic variability of expression and function of the multidrug resistance-associated proteins (MRPs, ABCC transporter) is to date rather limited as compared with eg, *MDR1*.[73] Outside the CNS, multiple but rare familial mutations in, eg, the ABCC2 gene (*MRP2*) are responsible for the recessive inherited Dubin-Johnson syndrome: although hepatic function is

normal, patients with this syndrome have an increased risk of drug-induced liver toxicity.[74]

Although SLCO transporters are genetically extensively characterized, relevant clinical data about the impact of polymorphisms are still limited. Genetic variants of uptake transporters have predominantly been investigated for OATPs, but a large number of single nucleotide polymorphisms in the OCT1 (*SLCO22A1*) and OCT2 (*SLCO22A2*) gene were also found, altering the transport function in vitro.[25,75] As OATP1A2 is predominantly localized in the capillary endothelial cells of the brain, genetic variability and polymorphisms of this drug uptake carrier may represent a future pathway for CNS drugs as it is a determinant of brain toxicity.[23]

Drug transporter interactions

Membrane transporters can undergo inhibition or induction, which respectively slow down or accelerate their transporter activity.[76-78]

In 1951, indirect clinical evidence already suggested the role of specific transport systems at the level of renal cell membranes[79]: coadministration of probenecid with penicillin resulted in decreased renal clearance, prolonged half-life, and elevated plasma level of penicillin, enabling a substantial reduction in antibiotic dose. The mechanism of this interaction was found several years later: the active penicillin secretion was reduced by OAT inhibition in the basolateral membrane of renal proximal tubule.[80] Similarly, coadministration of probenecid with HIV antiviral drugs or with antihypertensive drugs such as the angiotensin-converting enzyme inhibitors also causes a reduction in renal clearance, a prolonged half-life, and elevated plasma levels.[81] In humans, digoxin is a high-affinity substrate for MDR1, and most interacting drugs are either inductors, or, more frequently, inhibitors, of MDR1.[82] Significant MDR1 inhibition, by administrating atorvastatin, clarithromycin, or verapamil as MDR1 inhibitors, was associated with a significant increase in the serum digoxin concentration, ie, more

Symbol	Transporter	Polymorphism	Associated clinical effect
ABCB1	MDR1	3435 C>T	3435 C epilepsy drug-resistance[60] 3435 TT fewer viral resistance in efavirenz patients,[64] higher digoxin level,[65] and nortriptyline-induced postural hypotension[66]
ABCB1	MDR1	1236 C>T	1236 decreased survival in acute myeloid leukaemia patients[67]
ABCB1	MDR1	2677 G>T/A	2677 T tacrolimus-induced neurotoxicity[68]
ABCG2	BCRP	C421A	Increased plasma concentration of diflomotecan[69]
SCL21A3	OATP1A2	38T>C, 382A>T 404A>T, 516A>C 559 G>A, 2003 C>G	CNS penetration and toxicity of various drugs and substrates[52]
SLC22A6	OAT1	1361G>A, 877C>T 677T>C, 767C>T	Possible altered renal uptake of cidofovir and adefovir and drug-induced nephrotoxicity[70]
SLC22A1	OCT1	181C>T, 1201G>A	Decreased substrate uptake in vitro[71]
SLC22A2	OCT2	1198C>T, 495G>A	Decreased substrate uptake in vitro[72]

Table I. Examples of genetic polymorphisms in human drug transporters. ABC, adenosine triphosphate-binding cassette; MDR, multi-drug resistance; BCRP, breast cancer resistance protein; SLC, solute-linked carriers; OATP, organic anion transporting peptide; OAT, organic ion transporter; OCT, organic cation transporter; CNS, central nervous system.

Drug - substrate	Inhibitor / inducer	Interaction	Effect
Loperamide	Quinidine	MDR1 inhibition	Increased CNS penetration of loperamide[87]
Digoxin	Paroxetine	MDR1 inhibition	Increased CNS penetration of digoxin[75]
Cyclosporine	St John's wort	MDR1 induction	Decreased plasma level of cyclosporine[91]
Desipramine	Ritonavir	OCT1 inhibition	Decreased hepatic uptake with poorer access to CYP3A4[92]
Fexofenadine	Fruit juices	OATP inhibition	Decreased oral bioavailability[93]
Penicillin	Probenecid	OAT inhibition	Prolonged penicillin half-life by reduced renal clearance[81]

Table II. Examples of drug-drug interactions involving drug transporters. MDR, multidrug resistance; CNS, central nervous system; OCT, organic cation transporter; OATP, organic anion transporting peptide; OAT, organic ion transporter.

than twice the upper therapeutic limit.[76,78,83,84] Another striking and clinically relevant effect of the PGP-associated interactions was demonstrated by giving healthy volunteers loperamide, an opiate that is not absorbed from the gut, simultaneously with quinidine, a potent MDR1 inhibitor: coadministration of this antidiarrheal agent with quinidine resulted in central opioid effect such as respiratory depression and euphoria,[85,86] confirming in vivo a major MDR1 inhibition in the intestinal and in the BBB gatekeeper function.[52,87]

Recently, a population pharmacokinetic analysis of drug-drug interactions between risperidone, bupropion, and sertraline in rodents suggested that sertraline produces significant inhibitory effects on MDR1 transport at the BBB, increasing the brain entry of risperidone and its metabolite 9-OH-risperidone.[88] The order of magnitude was high, and could be clinically significant for humans: sertraline did not change the plasma concentration of risperidone and of its metabolite, but increased the brain area under the plasma concentration curve of risperidone and 9-hydroxy-risperidone 1.5-fold ($P<0.05$) and 5-fold ($P<0.01$), respectively.[88]

Interestingly, another study with rodents showed that the MDR1 localized in the BBB is more resistant to inhibition than in other tissues.[51] In vivo studies in humans are needed to assess the clinical relevance of such differential sensitivity to inhibition.

In vitro techniques for the assessment of drug-drug interactions involving membrane transporters are currently under development.[89] Inhibition on the MDR1 was investigated in vitro with recent antidepressants, displaying different interaction potentials: whereas paroxetine is regarded as a strong PGP inhibitor, citalopram and venlafaxine are considered as antidepressants with low interaction potential with membrane transporters.[77,90] A case report confirmed the ability of paroxetine to inhibit the PGP in the BBB and in the kidney, causing digitalis intoxication with delirium, visual hallucinations, and disorientation.[78] However, specific data from in vivo studies, substrate specificity, and inhibition or induction potential of psychiatric and neurological medication are still lacking. Examples related to drug-drug interaction at the membrane transporter level are illustrated in *Table II*.

Discussion

In vitro and in vivo studies show that drug carriers are expressed in the BBB and in the CSB. They represent major determinants of toxicity and clinical outcome related to drug response. Understanding the functional significance of membrane transporters in the BBB and in the CSB provides further opportunities to improve drug delivery to the CNS.

We propose that the role of transporter proteins should be studied at an early stage of CNS drug development, as there are in vitro methods such as cell cultures to achieve this purpose. Knockout animals are valuable in vivo models, but in vivo methods in humans are few. Direct in vivo determinations of drug concentration and effective transporter function into the brain remain particularly challenging, as invasive techniques are necessary. Neuroimaging techniques should be helpful, since molecules can be measured by positron emission tomography (PET) or by magnetic resonance imaging spectrometry. For example, the latter can be used to assess the pharmacokinetics of some fluoride-containing molecules in the brain.

Although several members of the membrane transporters present in the BBB have been characterized in detail, numerous questions remain open. Firstly, the determination of detailed tissue expressions and in vivo studies of carriers with better specificity are required to target more efficiently therapeutic agents into the CNS and into other organs. Secondly, in order to enhance the potential clinical implications of drug transporter polymorphisms and interactions, the development of specific inductors and inhibitors may represent promising strategies.

Thirdly, future delivery procedures include the use of prodrugs, drug-targeting vector conjugates, or liposomes tagged with targeting vectors to elude physiological barriers.

Drug transporter protein studies provide insight into the mechanisms of resistance, treatment failure, and interindividual response to neurological and psychiatric medication. Membrane transporter proteins are not only CNS gatekeepers, but represent determinant partners in CNS drug development strategies. Exploring the functional significance of membrane transporters in drug delivery to the CNS is essential for the treatment of neurological and psychiatric disorders. ❑

Proteínas transportadoras de membrana: un desafío para el desarrollo de fármacos para el sistema nervioso central

Los transportadores de fármacos son proteínas de membrana presentes en varios tejidos como linfocitos, intestino, hígado, riñón, testículos, placenta y el sistema nervioso central. Estos transportadores juegan un papel significativo en la absorción de fármacos y en la distribución a los sistemas del organismo, especialmente si los órganos están protegidos por barreras sangre-órgano, como la barrera hémato-encefálica o la barrera materno-fetal. En contraste con los neurotransmisores y los transportadores acoplados a receptores u otros modos de transmisión interneuronal, los transportadores de fármacos no están directamente involucrados en funciones neuronales específicas, pero proveen una protección global al sistema nervioso central. La falta de capilarización, la baja actividad de los pinocitos, y las uniones estrechas entre los capilares cerebrales y las células endoteliales de los plexos coroídeos representan más barreras que limitan la entrada de compuestos endógenos y exógenos al sistema nervioso central. El transporte de fármacos es el resultado de una acción concertada de bombas de entrada y salida (transportadores) ubicadas en las membranas basolaterales y apicales de los capilares cerebrales y de las células endoteliales de los plexos coroídeos. En la regulación de la entrada y salida de las sustancias endógenas o exógenas, la barrera hémato-encefálica, y en menor medida la barrera hémato-cerebroespinal en los ventrículos, representan el principal punto de contacto entre el sistema nervioso central y la sangre, es decir, el resto del organismo. Como la distribución de los fármacos a los órganos depende de la afinidad de un sustrato por un sistema de transporte específico, las proteínas transportadoras de membrana están siendo cada vez más reconocidas como un factor determinante en la disponibilidad de los fármacos. Muchos transportadores de fármacos son miembros de la superfamilia del transportador (ABC) unido a adenosín trifosfato (ATP) o de la clase de transportadores unidos a soluto (SLC). La proteína de resistencia a multifármacos MDR1 (ABCB1), también llamada P-glicoproteína, las proteínas asociadas a resistencia a multifármacos MRP1 (ABCC1) y MRP2 (ABCC2), y la proteína de resistencia al cáncer de mama BCRP (ABCG2) son transportadores de salida dependientes de ATP que se expresan en la barrera hémato-encefálica. Ellos pertenecen a la superfamilia de transportadores ABC, los cuales llevan fármacos desde el medio intracelular al extracelular. Los miembros de la clase SLC de los transportadores de soluto incluyen, por ejemplo, péptidos transportadores de iones orgánicos, transportadores de cationes orgánicos y transportadores de iones orgánicos. Ellos son polipéptidos independientes de ATP que se expresan principalmente en la membrana basolateral de los capilares cerebrales y las células endoteliales de los plexos coroídeos que también median el transporte de fármacos a través de las barreras del sistema nervioso central.

REFERENCES

1. Tamai I, Tsuji A. Transporter-mediated permeation of drugs across the blood-brain barrier. *J Pharm Sci*. 2000;89:1371-1388.
2. Kullak-Ublick GA, Fisch T, Oswald M, et al. Dehydroepiandrosterone sulfate (DHEAS): identification of a carrier protein in human liver and brain. *FEBS Lett*. 1998;424:173-176.
3. Kusuhara H, Sugiyama Y. Efflux transport systems for drugs at the blood-brain barrier and blood-cerebrospinal fluid barrier (Part 1). *Drug Discov Today*. 2001;6:150-156.
4. Kusuhara H, Sugiyama Y. Efflux transport systems for drugs at the blood-brain barrier and blood-cerebrospinal fluid barrier (Part 2). *Drug Discov Today*. 2001;6:206-212.
5. Vergani P, Basso C, Mense M, Nairn AC, Gadsby DC. Control of the CFTR channel's gates. *Biochem Soc Trans*. 2005;33(Pt 5):1003-1007.
6. Herget M, Tampe R. Intracellular peptide transporters in human - compartmentalization of the "peptidome." *Pflugers Arch*. 2006;May 18 [Epub ahead of print].
7. Pauli-Magnus C, Lang T, Meier Y, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics*. 2004;14:91-102.
8. Fernandez-Marmiesse A, Salas A, Vega A, Fernandez-Lorenzo JR, Barreiro J, Carracedo A. Mutation spectra of ABCC8 gene in Spanish patients with hyperinsulinism of infancy (HI). *Hum Mutat*. 2006;27:214.
9. Gueugnon F, Volodina N, Taouil JE, et al. A novel cell model to study the function of the adrenoleukodystrophy-related protein. *Biochem Biophys Res Commun*. 2006;341:150-157.
10. Bekri S, Kispal G, Lange H, et al. Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron-sulfur protein maturation. *Blood*. 2000;96:3256-3264.
11. Stenirri S, Battistella S, Fermo I, et al. De novo deletion removes a conserved motif in the C-terminus of ABCA4 and results in cone-rod dystrophy. *Clin Chem Lab Med*. 2006;44:533-537.
12. Michaelides M, Hardcastle AJ, Hunt DM, Moore AT. Progressive cone and cone-rod dystrophies: phenotypes and underlying molecular genetic basis. *Surv Ophthalmol*. 2006;51:232-528.
13. Cirrito JR, Deane R, Fagan AM, et al. P-glycoprotein deficiency at the blood-brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. *J Clin Invest*. 2005;115:3285-3290.
14. Stefkova J, Poledne R, Hubacek JA. ATP-binding cassette (ABC) transporters in human metabolism and diseases. *Physiol Res*. 2004;53:235-243.
15. Redzic ZB, Biringer J, Barnes K, et al. Polarized distribution of nucleoside transporters in rat brain endothelial and choroid plexus epithelial cells. *J Neurochem*. 2005;94:1420-1426.

Protéines de transport membranaires : un défi pour le développement des médicaments du SNC

Les transporteurs de médicaments (drug transporters) *sont des protéines situés dans les membranes cellulaires de différents tissus comme les lymphocytes, l'intestin, le foie, les reins, les testicules, le placenta ou le SNC (système nerveux central). Ces transporteurs jouent un rôle primordial dans l'absorption et la distribution des médicaments dans les organes cibles, surtout s'ils sont protégés par des structures comme la barrière hématoencéphalique (BHE) ou la barrière maternofœtale. Contrairement aux transporteurs liés à des récepteurs ou à la transmission interneuronale, ces transporteurs membranaires ne sont pas directement impliqués dans une fonction neuronale spécifique mais assurent une protection globale du système nerveux central. Le transport de médicaments est la résultante des mécanismes d'efflux (sortie) et d'influx (entrée) situés au niveau de la membrane apicale et basolatérale des cellules endothéliales des capillaires cérébraux et du plexus choroïdien. En régulant l'efflux et l'influx des substances endogènes ou exogènes, la BHE et la barrière hémato-cérébro-spinale représentent les principales interfaces entre le système nerveux central et le sang. La distribution d'un médicament vers les organes cibles est dépendante de son affinité pour un système de transport spécifique : les transporteurs membranaires sont ainsi reconnus comme un élément clé dans la disposition des médicaments au niveau du système nerveux central. Plusieurs transporteurs membranaires font partie de la superfamille ABC* (ATP-Binding Cassette) *ou SLC* (Solute Carriers)*. La* Multi-Drug Resistance protein *MDR1 (ABCB1) nommée aussi P-glycoprotéine, les* Multidrug Resistance-associated Proteins *MRP1 (ABCC1) et MRP2 (ABCC2), ainsi que la* Breast Cancer Resistance Protein *BCRP (ABCG2) sont des transporteurs d'efflux ATP-dépendants présents au niveau de la BHE et qui appartiennent à la superfamille des transporteurs ABC. Les OATPs* (Organic Anion Transporter Proteins)*, OCTs* (Organic Cation Transporters) *et OATs* (Organic Anion Transporters) *font partie des transporteurs ATP-indépendants de type SLC constitués de polypeptides* (solute carriers)*. Les conséquences cliniques des transporteurs membranaires semblent jouer un rôle de plus en plus important dans le développement de nouveaux médicaments, particulièrement en neurologie et en psychiatrie. Les différents mécanismes qui régulent le transport de molécules à travers les membranes cellulaires et les barrières organiques sont des éléments clés pour expliquer les mécanismes d'échec ou de résistance au traitement, ainsi que certains effets indésirables et variations individuelles à la réponse thérapeutique attendue.*

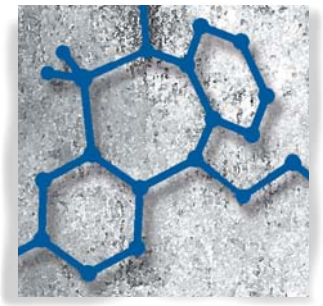
16. Steuer H, Jaworski A, Elger B, et al. Functional characterization and comparison of the outer blood-retina barrier and the blood-brain barrier. *Invest Ophthalmol Vis Sci*. 2005;46:1047-1053.
17. Hawkins RA, Peterson DR, Vina JR. The complementary membranes forming the blood-brain barrier. *IUBMB Life*. 2002;54:101-107.
18. Ganong WF. Circumventricular organs: definition and role in the regulation of endocrine and autonomic function. *Clin Exp Pharmacol Physiol*. 2000;27:422-427.
19. Strazielle N, Khuth ST, Ghersi-Egea JF. Detoxification systems, passive and specific transport for drugs at the blood-CSF barrier in normal and pathological situations. *Adv Drug Deliv Rev*. 2004;56:1717-1740.
20. Emerich DF, Vasconcellos AV, Elliott RB, Skinner SJ, Borlongan CV. The choroid plexus: function, pathology and therapeutic potential of its transplantation. *Expert Opin Biol Ther*. 2004;4:1191-201.
21. Ito K, Suzuki H, Horie T, Sugiyama Y. Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport. *Pharm Res*. 2005;22:1559-1577.
22. Ghersi-Egea JF, Strazielle N. Choroid plexus transporters for drugs and other xenobiotics. *J Drug Target*. 2002;10:353-357.
23. Hagenbuch B, Gao B, Meier PJ. Transport of xenobiotics across the blood-brain barrier. *News Physiol Sci*. 2002;17:231-234.
24. Loscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol*. 2005;76:22-76.
25. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. *Cancer Lett*. 2006;234:4-33.
26. Taylor EM. The impact of efflux transporters in the brain on the development of drugs for CNS disorders. *Clin Pharmacokinet*. 2002;41:81-92.
27. Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog*. 1995;13:129-134.
28. Kusuhara H, Sugiyama Y. Active efflux across the blood-brain barrier: role of the solute carrier family. *NeuroRx*. 2005;2:73-85.
29. Oswald C, Holland IB, Schmitt L. The motor domains of ABC-transporters. What can structures tell us? *Naunyn Schmiedebergs Arch Pharmacol*. 2006;372:385-399.
30. Leslie EM, Deeley RG, Cole SP. Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol.* 2005;204:216-237.
31. Leier I, Jedlitschky G, Buchholz U, Cole SP, Deeley RG, Keppler D. The MRP gene encodes an ATP-dependent export pump for leukotriene C4 and structurally related conjugates. *J Biol Chem.* 1994;269:27807-27810.
32. Leier I, Jedlitschky G, Buchholz U, et al. ATP-dependent glutathione disulphide transport mediated by the MRP gene-encoded conjugate export pump. *Biochem J.* 1996;314(Pt 2):433-437.
33. Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther*. 2005;78:260-277.

34. Soontornmalai A, Vlaming ML, Fritschy JM. Differential, strain-specific cellular and subcellular distribution of multidrug transporters in murine choroid plexus and blood-brain barrier. *Neuroscience*. 2006;138:159-169.
35. Calatozzolo C, Gelati M, Ciusani E, et al. Expression of drug resistance proteins Pgp, MRP1, MRP3, MRP5 and GST-pi in human glioma. *J Neurooncol*. 2005;74:113-121.
36. Bronger H, Konig J, Kopplow K, et al. ABCC drug efflux pumps and organic anion uptake transporters in human gliomas and the blood-tumor barrier. *Cancer Res*. 2005;65:11419-11428.
37. Kubota H, Ishihara H, Langmann T, et al. Distribution and functional activity of P-glycoprotein and multidrug resistance-associated proteins in human brain microvascular endothelial cells in hippocampal sclerosis. *Epilepsy Res*. 2006;68:213-228.
38. Toornvliet R, van Berckel BN, Luurtsema G, et al. Effect of age on functional P-glycoprotein in the blood-brain barrier measured by use of (R)-[C]verapamil and positron emission tomography. *Clin Pharmacol Ther*. 2006;79:540-548.
39. Sasongko L, Link JM, Muzi M, et al. Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. *Clin Pharmacol Ther*. 2005;77:503-514.
40. Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch*. 2004;447:653-665.
41. Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Introduction. *Pflugers Arch*. 2004;447:465-468.
42. Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta*. 2003;1609:1-18.
43. Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ. Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol Endocrinol*. 2002;16:2283-2296.
44. Galic S, Schneider HP, Broer A, Deitmer JW, Broer S. The loop between helix 4 and helix 5 in the monocarboxylate transporter MCT1 is important for substrate selection and protein stability. *Biochem J*. 2003;376(Pt 2):413-422.
45. Halestrap AP, Meredith D. The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch*. 2004;447:619-628.
46. Enerson BE, Drewes LR. Molecular features, regulation, and function of monocarboxylate transporters: implications for drug delivery. *J Pharm Sci*. 2003;92:1531-1544.
47. Bhattacharya I, Boje KM. GHB (gamma-hydroxybutyrate) carrier-mediated transport across the blood-brain barrier. *J Pharmacol Exp Ther*. 2004;311:92-98.
48. Busch AE, Karbach U, Miska D, et al. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol Pharmacol*. 1998;54:342-352.
49. van Montfoort JE, Muller M, Groothuis GM, Meijer DK, Koepsell H, Meier PJ. Comparison of "type I" and "type II" organic cation transport by organic cation transporters and organic anion-transporting polypeptides. *J Pharmacol Exp Ther*. 2001;298:110-115.
50. Evans WE, McLeod HL. Pharmacogenomics - drug disposition, drug targets, and side effects. *N Engl J Med*. 2003;348:538-549.
51. Choo EF, Kurnik D, Muszkat M, et al. Differential in vivo sensitivity to inhibition of P-glycoprotein located in lymphocytes, testes, and the blood-brain barrier. *J Pharmacol Exp Ther*. 2006;317:1012-1018.
52. Lee W, Glaeser H, Smith LH, et al. Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J Biol Chem*. 2005;280:9610-9617.
53. O'Kane DJ, Weinshilboum RM, Moyer TP. Pharmacogenomics and reducing the frequency of adverse drug events. *Pharmacogenomics*. 2003;4:1-4.
54. Lin JH, Yamazaki M. Clinical relevance of P-glycoprotein in drug therapy. *Drug Metab Rev*. 2003;35:417-454.
55. Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet*. 2003;42:59-98.
56. Schinkel AH, Smit JJ, van Tellingen O, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell*. 1994;77:491-502.
57. Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. *Annu Rev Pharmacol Toxicol*. 2003;43:285-307.
58. Babaoglu MO, Bayar B, Aynacioglu AS, et al. Association of the ABCB1 3435C>T polymorphism with antiemetic efficacy of 5-hydroxytryptamine type 3 antagonists. *Clin Pharmacol Ther*. 2005;78:619-626.
59. Eichelbaum M, Fromm MF, Schwab M. Clinical aspects of the MDR1 (ABCB1) gene polymorphism. *Ther Drug Monit*. 2004;26:180-185.
60. Siddiqui A, Kerb R, Weale ME, et al. Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. *N Engl J Med.* 2003;348:1442-1448.
61. Weinshilboum R. Inheritance and drug response. *N Engl J Med*. 2003;348:529-537.
62. Crivori P, Poggesi I. Computational approaches for predicting CYP-related metabolism properties in the screening of new drugs. *Eur J Med Chem*. 2006;Apr 24 [Epub ahead of print].
63. Konig J, Seithel A, Gradhand U, Fromm MF. Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol*. 2006;372:432-443.
64. Haas DW, Smeaton LM, Shafer RW, et al. Pharmacogenetics of long-term responses to antiretroviral regimens containing efavirenz and/or nelfinavir: an adult AIDS Clinical Trials Group Study. *J Infect Dis*. 2005;192:1931-1942.
65. Verstuyft C, Schwab M, Schaeffeler E, et al. Digoxin pharmacokinetics and MDR1 genetic polymorphisms. *Eur J Clin Pharmacol*. 2003;58:809-812.
66. Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenomics J.* 2002;2:191-196.
67. Illmer T, Schuler US, Thiede C, et al. MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res*. 2002;62:4955-4962.
68. Yamauchi A, Ieiri I, Kataoka Y, et al. Neurotoxicity induced by tacrolimus after liver transplantation: relation to genetic polymorphisms of the ABCB1 (MDR1) gene. *Transplantation*. 2002;74:571-572.
69. Sparreboom A, Gelderblom H, Marsh S, et al. Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. *Clin Pharmacol Ther*. 2004;76:38-44.
70. Fujita T, Brown C, Carlson EJ, et al. Functional analysis of polymorphisms in the organic anion transporter, SLC22A6 (OAT1). *Pharmacogenet Genomics*. 2005;15:201-209.
71. Kerb R, Brinkmann U, Chatskaia N, et al. Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. *Pharmacogenetics*. 2002;12:591-595.
72. Leabman MK, Huang CC, Kawamoto M, et al. Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics*. 2002;12:395-405.
73. Dallas S, Miller DS, Bendayan R. Multidrug resistance-associated proteins: expression and function in the central nervous system. *Pharmacol Rev*. 2006;58:140-161.
74. Elferink RO, Groen AK. Genetic defects in hepatobiliary transport. *Biochim Biophys Acta*. 2002;1586:129-145.
75. Marzolini C, Tirona RG, Kim RB. Pharmacogenomics of the OATP and OAT families. *Pharmacogenomics*. 2004;5:273-282.
76. Wakasugi H, Yano I, Ito T, et al. Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. *Clin Pharmacol Ther* 1998;64:123-128.
77. Weiss J, Dormann SM, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash N, Haefeli WE. Inhibition of P-glycoprotein by newer antidepressants. *J Pharmacol Exp Ther*. 2003;305:197-204.
78. Yasui-Furukori N, Kaneko S. Digitalis intoxication induced by paroxetine co-administration. *Lancet*. 2006;367:788.
79. Burnell JM, Kirby WM. Effectiveness of a new compound, benemid, in elevating serum penicillin concentrations. *J Clin Invest*. 1951;30:697-700.
80. Jariyawat S, Sekine T, Takeda M, et al. The interaction and transport of beta-lactam antibiotics with the cloned rat renal organic anion transporter 1. *J Pharmacol Exp Ther.* 1999;290:672-677.
81. Ayrton A, Morgan P. Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica*. 2001;31:469-497.
82. Parker RB, Yates CR, Soberman JE, Laizure SC. Effects of grapefruit juice on intestinal P-glycoprotein: evaluation using digoxin in humans. *Pharmacotherapy*. 2003;23:979-987.
83. Aszalos A, Thompson K, Yin JJ, Ross DD. Combinations of P-glycoprotein blockers, verapamil, PSC833, and cremophor act differently on the multidrug resistance associated protein (MRP) and on P-glycoprotein (Pgp). *Anticancer Res*. 1999;19:1053-1064.

84. Boyd RA, Stern RH, Stewart BH, et al. Atorvastatin coadministration may increase digoxin concentrations by inhibition of intestinal P-glycoprotein-mediated secretion. *J Clin Pharmacol*. 2000;40:91-98.
85. Eneroth A, Astrom E, Hoogstraate J, et al. Evaluation of a vincristine resistant Caco-2 cell line for use in a calcein AM extrusion screening assay for P-glycoprotein interaction. *Eur J Pharm Sci*. 2001;12:205-214.
86. Frohlich M, Albermann N, Sauer A, Walter-Sack I, Haefeli WE, Weiss J. In vitro and ex vivo evidence for modulation of P-glycoprotein activity by progestins. *Biochem Pharmacol*. 2004;68:2409-2416.
87. Sadeque AJ, Wandel C, He H, Shah S, Wood AJ. Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin Pharmacol Ther*. 2000;68:231-237.
88. Wang JS, DeVane CL, Gibson BB, Donovan JL, Markowitz JS, Zhu HJ. Population pharmacokinetic analysis of drug-drug interactions among risperidone, bupropion, and sertraline in CF1 mice. *Psychopharmacology* (Berl). 2006;183:490-499.
89. Keogh JP, Kunta JR. Development, validation and utility of an in vitro technique for assessment of potential clinical drug-drug interactions involving P-glycoprotein. *Eur J Pharm Sci*. 2006;27:543-554.
90. Koren G, Woodland C, Ito S. Toxic digoxin-drug interactions: the major role of renal P-glycoprotein. *Vet Hum Toxicol*. 1998;40:45-46.
91. Leabman MK, Huang CC, Kawamoto M, et al. Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics*. 2002;12:395-405.
92. Dresser GK, Schwarz UI, Wilkinson GR, Kim RB. Coordinate induction of both cytochrome P4503A and MDR1 by St John's wort in healthy subjects. *Clin Pharmacol Ther*. 2003;73:41-50.
93. Dresser GK, Spence JD, Bailey DG. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet*. 2000;38:41-57.

Experimental animal models for the simulation of depression and anxiety

Eberhard Fuchs, PhD; Gabriele Flügge, PhD



Animal models of psychiatric diseases attempt to capture various features of the human condition, from behavioral and physiological changes that are indicative of the emotional state to the etiology of the disease and the effects of therapeutic interventions. According to McKinney,[1] animal models are "experimental preparations developed in one species for the purpose of studying phenomena occurring in another species. In the case of animal models of human psychopathology one seeks to develop syndromes in animals which resemble those of humans in certain ways in order to study selected aspects of human psychopathology." Later, other authors (eg, ref 2) proposed additional criteria that animal models need to fulfill. Suitable research models ought to display clear *face validity* (isomorphism), *predictive validity* (pharmacological correlation), and *construct validity*

An impressive number of animal models to assess depression and anxiety are available today. However, the relationship between these models and the clinical syndromes of depression and anxiety is not always clear. Since human anxiety disorders represent a multifactorial phenomenon frequently comorbid with major depression and/or other psychiatric problems, the chance of creating animal models which consistently reflect the human situation is quite poor. When using experimental models to understand homologies between animal and human behavior, we have to consider the context in which an animal is investigated, and both the functional significance and relevance of the behavioral parameters that are quantified. Moreover, gender and interindividual and interspecies variabilities in behavioral responses to the test situation and in the sensitivity to pharmacological treatments are potential sources for confounding results. In the past, these aspects have been often neglected in preclinical approaches to behavioral pharmacology and psychopharmacology. A pragmatic approach of combined preclinical and clinical efforts is necessary to imitate one or more aspects relevant to pathological anxiety disorders and depression. The resulting models may identify central nervous processes regulating defined behavioral output, with the potential to develop more effective treatments.

Dialogues Clin Neurosci. 2006;8:323-333.

Keywords: *affective disorder; chronic drug treatment; glucocorticoid; normal anxiety; pathological anxiety; gender difference; rat; mouse*

Author affiliations: Clinical Neurobiology Laboratory, German Primate Center, Göttingen, Germany (Eberhard Fuchs, Gabriele Flügge); Department of Neurology, Medical School, University of Göttingen, Germany (Eberhard Fuchs)

Address for correspondence: Prof Eberhard Fuchs, Clinical Neurobiology Laboratory, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany (e-mail: efuchs@gwdg.de)

(homology and similarity in the underlying neurobiological mechanisms). Currently, the third criterion is regarded as having heuristic value because the central nervous processes that lead to anxiety/depression still have to be elucidated; therefore this criterion is regarded as desirable, but not essential.[3] Thus, in an ideal and perfect model one would like to have causative conditions, symptom profiles, and treatment responses identical to those seen in the human disease state.

Any animal model of depression, or of antidepressant activity, must account for the considerable symptom overlap between major depressive disorder (MDD) and anxiety disorders, eg, sleep disturbances, agitation, restlessness, irritability, difficulty concentrating, loss of control, fatigue, fear, distress and, of course, anxiety. Indeed, comorbidity of anxiety disorders and MDD is the rule rather than the exception (eg, refs 4-6)with more than 80% of adults with depression also having significant symptoms of anxiety.[7] Furthermore, most of the existing antidepressants successfully ameliorate anxiety as a component of depression (eg, ref 8).

In this article we will discuss relevant animal models that have been developed and are used to enhance our understanding of the pathophysiology of the most common psychiatric disorders, depression and anxiety, and to guide the development of novel and more effective treatments.

Animal models of depression

The diagnosis of depressive illness and anxiety relies almost exclusively on observation of behavior and interpersonal relations, and on reported feelings and beliefs of the patient.[9] Therefore, several recent reviews claim that it is difficult to develop a true animal model of depressive disorders because mental illness may be a uniquely human condition. In particular, typical symptoms in depressed patients, such as recurring thoughts of suicide or death, or excessive thoughts of guilt, are impossible to model in animals. The creation of reasonably valid animal models of psychiatric diseases has been difficult, mainly due to both the verbal and personal nature of the symptoms to be modeled, eg, sadness or delusions, as well as the lack of clear etiological factors which can be used to design valid models. Moreover, unlike the situation with other neurological disorders such as Alzheimer's disease or Parkinson's disease, we still have only a vague idea about the pathophysiological processes that underlie depression.

The earliest models of depressive states in animals were based on *maternal separation experiments* in infant nonhuman primates.[10] In rodents, manipulation of early life environment such as *prenatal stress* and maternal separation produces bio behavioral changes that persist well into adulthood, representing a risk factor for psychopathology.[11] Another behavioral approach to simulate a human depressive state in animals is the *learned helplessness* model. Originally described in dogs subjected to inescapable electric shock,[12] this model has received considerable attention in studies of "depression" in mice and rats (for review see refs 13, 14). Limitations of the helplessness test as consequence of foot shock are that the test is difficult to replicate between laboratories[15] and that it cannot be routinely used in a number of countries because of ethical or regulatory supervision.[14]

The *chronic mild stress* model is based on exposure of animals (usually rats) to uncontrollable stressors. Animals are subjected, in succession, to a range of mild stressors such as disrupted light-dark cycle, wet bedding, having an intruder rat placed in the home cage, or having the home cage tilted at an angle for 1 to 2 days.[16] The complex procedures of this model almost ensure that every laboratory will have at least slightly different experimental setups, and consequently, also different interpretations of the protocols.[14]

Among the most potent factors known to trigger or induce depressive episodes are stressful life events.[17-20] Stress is considered to perturb the homeostasis of an organism in a way that can lead to a long-lasting imbalance in neurotransmitter, neuroendocrine, and hormonal systems and thus finally to a psychiatric disease. The stress hypothesis of mood disorders has stimulated the development of a number of putative animal models of depression.[2,21] Loss of rank and/or social status in humans is one example of loss experiences which are increasingly recognized as specific type of "life event" associated with a great risk of depression.[22] A number of behavioral models have sought to stimulate or model depression by manipulating social relationships in animals, and new powerful animal models using chronic psychosocial perturbations as stressors have been established (eg, ref 23). In recent years, our group has provided increasing evidence that chronic psychosocial stress in the male tree shrew (*Tupaia belangeri*) represents a natural and valid paradigm for studying the behavioral, endocrine, and neurobiological changes that may underlie stress-related disorders such as depression.[24] Recently, our group has

described and validated a new model of chronic social stress in rats[25] based on the resident–intruder paradigm originally described by Miczek[26] and Koolhaas et al.[27] This model, in which depressive-like behavior can be normalized by antidepressants, provides the opportunity to study gene expression in distinct brain areas.[28,29] Although the relevant literature is constantly expanding, one can already summarize today that models of social stress greatly increased our understanding of processes that take place in the brain during depressive-like states of an animal. Also the understanding of antidepressant-induced processes has greatly increased in the past years (eg, ref 30).

A summary of animal models of depression that are classified according to type and sensitivity to chronic drug treatment is presented in *Table I*. According to Willner and Mitchell,[31] the *diathesis models* summarize those paradigms that involve a genetically determined predisposition for the depressive illness, whereas in mere *stress models* external stimuli are the only factors triggering changes in behavior and physiology. *Social dominance models* are those that use natural (social) stressors and are considered as a subset of the stress models.

Many of the paradigms addressed above are more correctly described as models of stress rather than models of depression. Not all responses to stress are maladaptive, because the stress response may also fulfill adaptive or protective functions. Therefore, to truly model depression, other factors such as the genetic background that might cause a predisposition for the disease must also be taken into consideration. However, studies looking at stressful early life experiences and the type of stress-responsiveness later in life highlight a key area. They may help to understand the processes that in conjunction with environmental stress can lead to depression in some individuals but not in others.

Model type	Sensitive to chronic drug treatment
Diathesis models	
Lesion model	Olfactory bulbectomy
Genomic model	HPA axis transgenes
Genetic models	Flinders sensitive line
Developmental models	Prenatal or neonatal stress
Stress models	
Acute stress	Learned helplessness Restraint stress
Chronic stress	Chronic mild stress Restraint stress
Social dominance models	
Social separation	Neonatal / adult isolation
Social defeat	Resident-intruder Social hierarchy

Table I. Animal models of depression.

With the emergence of specific genetic factors more defined models may be created in the near future. In the case of major depressive illness, we know that genetic factors can only account for about 30% of the variance, and environmental factors clearly play a major role in inducing the illness.[32] However, the development of models of depression based on the interaction between stress and genetic vulnerability appears plausible. Generation of specific strains or lines of rats or mice may be advantageous. Studies in knockout models with a mutation in a single gene may be of limited usefulness because of confounding factors such as developmental adaptational processes. Conditional knockouts may be considered as an improvement, but they also can inform us only about the role of a single gene. Therefore, the more complex models involving the interaction of *genes and environment* could supposedly yield more useful information.

Validity of animal models

The importance of chronic drug treatment

Pharmacological tests and models sensitive to acute drug treatment are not included in this overview. These models, perhaps more appropriately called "screens," [33] have been designed to detect most existing antidepressants. The mechanism(s) of action by which test compounds produce positive results in such screens may not be identical, or even not similar to the mechanisms underlying their clinical effects. One might have concerns about how drugs are applied to animals in preclinical experiments when comparing the routes of administration with those generally used in clinical settings. Many screens try to detect antidepressant-like activity quite quickly, within minutes or hours, and the drugs are given prior to the testing, thus producing a behavioral alteration rather than preventing a disease-induced type of behavior. It is obvious that such an approach bears no similarity to the clinical situation where drugs are administered only *after disease symptoms* have already appeared, and where a delayed onset of therapeutic effects for at least 2 to 3 weeks has to be expected. In light of such data, we would suggest that one important characteristic factor for ani-

mal models with predictive validity is the reproduction of a *time course* of the "therapeutic effects."
Furthermore, in most studies drugs are given intraperitoneally (IP) instead of orally, although the oral administration provides several advantages: (i) it mimics the clinical situation, where most patients take the drug orally; (ii) drugs taken orally produce metabolite concentrations that differ from those obtained after IP or intravenous (IV) administration; and (iii) it minimizes the uncontrollable stress effects of injections.
Also, little attention has so far been paid to potential species-specific differences in the metabolism of the applied drugs and their dosages. To exclude the effects of sub- or supraeffective doses there is an urgent need for monitoring the concentrations of circulating antidepressants and their pharmacologically active metabolites in the animals to be tested in the studies. Equally important is the observation that the drug effects are seen at clinically relevant doses that do not produce other, potentially confounding effects on physiology and behavior.[34]
The clinical requirement for chronic treatment regimes has produced extensive literature describing the effects of chronic antidepressant treatment in normal animals without paying attention to the basal state of targeted neural systems. Administration of antidepressant medication, or electroconvulsive stimulation, to nondepressed humans almost certainly does not elicit the same neural changes as when applied to a depressed patient. Therefore, we should make sure that the basal state of laboratory animals undergoing trials of (putative) antidepressants closely mirrors what is known about the neural changes that occur in depressed humans.
As outlined above, the ideal model should respond to chronic, but not acute, treatment with conventional antidepressants. The importance of this feature should not be underestimated, since only when a model shows a gradual response reflecting a drug's gradual onset of action is it possible to detect the actual time point of the therapeutic onset. Two models for which the clearest evidence for gradual onset of action has been obtained are the chronic mild stress model and the social stress/resident-intruder paradigm.
It is well known that the environment plays an important role in determining behavior and, eg, lighting conditions and familiarity of the experimental settings have a profound impact on the behavior that an experimental animal displays. Therefore, it is a major problem that in the laboratory, the "daytime" when an animal's behavior is observed is determined purely by the experimenter. It is quite frequently neglected that laboratory rodents are nocturnal, and thus generally quiescent during the light phase of the day. Therefore, in rodents determination of the effect of psychotropic drugs on natural action patterns of behavior should be performed during the dark phase of the light-dark cycle. This means that animals must be housed under a reversed light-dark schedule.[34]

Glucocorticoids and depression

Major depressive disorder is a complex, multifactorial and heterogeneous mental disorder[9] and its phenotypic heterogeneity requires the development of "multi-phenomenon" animal models. As an example of problematic clinical heterogeneity and its impact upon the utility of animal modeling, we will briefly discuss the hypothesis of hypercortisolism that has been widely considered as one of the fundamental neurobiological abnormalities of depression, and thus has dominated the relevant literature for many years.
If we are developing or using a valid animal model based upon perturbed corticosteroid function as a core aspect of depression, we must be confident that such perturbation is a reliable feature of the clinical presentation of depression. However, the clinical situation reveals that depressed subjects show a remarkable heterogeneity of neuroendocrine functions and that patients with hypothalamo–pituitary–adrenal (HPA) axis hyperactivity during acute depression may be in the range of only 35%.[35] Interestingly, hypercortisolism has also been described in patients with quite different diagnoses such as Alzheimer's disease[36] or substance abuse.[37]
A recent study by Strickland et al[38] in women revealed that, although well-defined adverse life events were associated with increased cortisol concentrations in saliva, depression was not. In light of these and other findings in patients, Matthews et al[35] posed the question of the validity and relevance of studies modeling depression in animals with the focus predominantly on corticosteroid function and regulation. However, although these data are not incompatible with the theory that stress predisposes to depression through its effects on the HPA axis, one cannot exclude that pre-existing HPA-axis abnormalities represent a contributory factor in the genesis of some forms of depression.

Animal models of anxiety

Anxiety enables the individual to recognize danger and to deal with an unknown or vague internal or external threat. Fear is a similar alerting signal, but differs from anxiety in that it is regarded as response to a known, definite, nonconflictual threat. Clinicians assessing anxiety distinguish between "normal" and "pathological" anxiety. Normal anxiety is an advantageous response to a threatening situation that accompanies many aspects of daily life. By contrast, pathological anxiety is an inappropriate response to an external or internal stimulus.
In light of the high complexity of anxiety disorders and the comorbidity with MDD, the chance of succeeding in developing comprehensive animal models that accurately reflect the relative influences of contributing factors in humans is probably quite poor.[39] However, as outlined below, ample opportunity exists to better define and extend existing models and to develop new experimental setups that consider the impact of combined factors in determining anxious behavior. The examples summarized in this part of the article have been selected because they model cardinal symptoms of anxiety but not depressive disorders.

Validity criteria for animal models of anxiety disorders

Numerous procedures with experimental animals have been developed to facilitate preclinical research on the behavioral pharmacology of anxiety and, as a result of this aim, are often referred to as "animal models of anxiety." This is an unfortunate error in terminology, not only because it implies that anxiety is a unitary emotional state, but also because of the apparent inability of many tests to consistently detect the anxiolytic effects of novel drugs.[40] The discovery of benzodiazepines (BZs) about 50 years ago, and their therapeutic and commercial success in the treatment of anxiety, has stimulated the development of a number of experimental test procedures. Because BZs were the only effective anxiolytic drugs at that time, the predictive validity of the animal models has been mainly based on their ability to detect the pharmacological action of BZs and related compounds. Later, clinicians discovered that patients can become addicted to BZ, and consequently paid more attention to non-benzodiazepine anxiolytics. However, it turned out that these new drugs were a challenge to the validity of the existing screening models. The best known example is buspirone, a clinically effective serotonin $(5\text{-HT})_{1A}$ receptor partial agonist whose anxiolytic potential was missed by conventional screening procedures in animals, in particular conflict tests in rats, and was only recognized during clinical assessments for possible antipsychotic efficacy.[41] This was the time when unconditioned conflict tests such as the elevated plus-maze were developed.[42]
A further complication appeared when it became evident that anxiety is not a unitary phenomenon, but could be divided into various forms including "normal" or "state" anxiety on the one hand and "pathological" or "trait" anxiety on the other hand. According to today's terminology, pathological anxiety should not be considered just as an excess of normal anxiety, but it rather appears that the pathological forms have a different neurobiological basis. Furthermore, the various forms of human anxiety disorders have been shown to be differentially sensitive to pharmacological treatment.
Most of the experimental paradigms involve exposure of animals to external stimuli (eg, cues paired with foot shock, bright light for rodents, or exposure to a predator) or internal stimuli (eg, drugs) that are assumed to induce anxiety. Because none of these models involves *pathological* anxiety, that is an anxiety-like state independent of an obvious (external) stimulus, Lister[43] described them as animal models of state anxiety. In these experimental set-ups, subjects experience normal anxiety at a particular moment in time and their emotional state is just potentiated by an external anxiogenic stimulus.
Despite these problems in the use of animals to study anxiety, these models have been, and are still, indispensable for neurobiological/neuropharmacological research. Much of our understanding of the neural substrates of anxiety has emerged from studies employing animal models that emulate aspects of the presumed etiology, physiology, and behavioral expression of fear and anxiety. There are several excellent book chapters and review articles describing and discussing extensively these models.[2,39,40,43-46] However, a survey of current literature reveals a confusing diversity of experimental procedures with more than 30 behavioral paradigms claiming face, construct, and/or predictive validity as animal models of anxiety disorders (for review see refs 47-49).

Models for normal anxiety

An overview of the existing models for *normal anxiety* is given in *Figure 1.* As proposed by Griebel[47] these models

are distinguished according to the following categories: (i) Models based on unconditioned responses; and (ii) models based on conditioned responses. The first category is further divided into four subgroups: models based on exploratory behavior in rodents (eg, *elevated plus-maze* and the *light-dark test*), models based on social behavior in rodents (*social interaction test*) or in non-human primates (*human threat*), and models based on somatic stress reactions (eg, *stress-induced hyperthermia*). In the fourth group, other paradigms are summarized which do not fit easily into the other subgroups such as the *anxiety/fear test battery*.

Elevated plus-maze

Today, the majority of studies using animal models of normal or state anxiety employ unconditioned-based procedures that rely on the natural behavior of the animals. Among these, the *elevated plus-maze* has become one of the most popular behavioral tests.[42,48] Its popularity is mainly due to practical reasons, because the elevated plus-maze permits a quick screening of potential anxiety-modulating drugs or of genetically modified laboratory rodents without training the animals or involvement of complex schedules.[48] The elevated maze consists of two opposite open and two closed alleys. When the animal is taken straight from its home cage it explores the different alleys and the total number of entries is counted. Anxiolytics help to overcome the fear-induced inhibition of open-alley exploration, while anxiogenic agents suppress open-alley exploration. Unfortunately, the plus-maze behavior patterns may be influenced by variations in test parameters that are not always obvious, eg, the species or strain investigated, housing conditions,

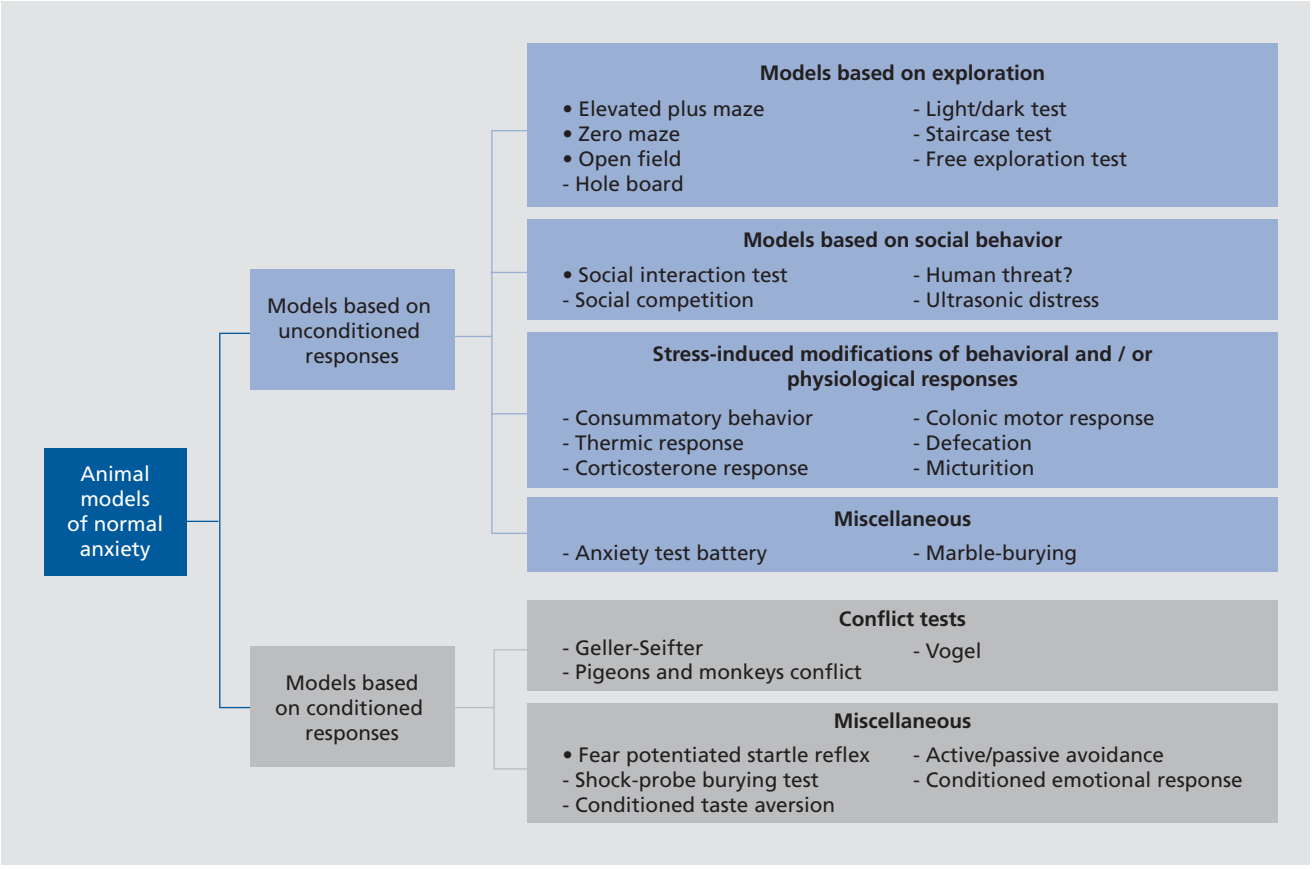


Figure 1. Classification of the existing animal models for normal or state anxiety.[46,47] For reasons of clarity, models are placed into one of the following two categories: Tests based on unconditioned responses and tests based on conditioned responses. Tests described in this article are marked by •.

day time of the testing, intensity of the light, and scoring method.[50] As a result, a vast number of studies employing the elevated plus-maze have yielded inconsistent findings. To overcome these problems, Rodgers and Johnson[51] have developed an "ethological" version of the mouse plus-maze that incorporates species-specific behavioral postures (eg, risk assessment, head-dipping) together with the conventional spatiotemporal measures of open-arm avoidance.

Elevated zero maze

This is a recent modification of the plus-maze designed for investigations in mice. It is an elevated annular platform with two opposite open and two closed quadrants. Animals are placed in one of the closed quadrants designated as the starting quadrant and anxiety related behaviors are recorded by both the observer and through a video system.

Open field test

Rodents are night-active animals that prefer darkness and avoid bright areas. This has to be taken into account when using the *open field test*, a very common observational method.[52] For the open field test, the animal is taken from its home cage and placed in a novel and relatively lit arena that is large enough for the animal to move around in. The area is divided into peripheral and central units, and locomotion and rearing can be recorded in these units. Because of its photophobicity, the animal avoids the brightly lit open spaces and prefers to stay close to the walls. Exploratory or locomotor behavior is therefore measured while determining the distance from the wall, and autonomic activity such as urination and defecation is evaluated. By using infrared beam array systems, locomotion, rearing and time spent in certain predefined areas of the open field are measured automatically. One also has to consider that the behavior displayed in the open field—similar to that in the elevated plus maze—is remarkably sensitive to a variety of internal and external factors.

Social interaction test

The *social interaction* test that was originally introduced by File,[53] and that quantifies the level of social behavior between animals, is a valuable behavioral paradigm for testing anxiolytic drugs. Experimental animals unfamiliar to each other are placed in pairs into an open arena. When the arena is brightly illuminated the situation is aversive for the animals, so that they reduce their social interactions. Anxiolytics usually increase the time spent in social interactions.

Fear-potentiated startle test

Davis and colleagues[54] have utilized the *fear-potentiated startle test* to study the fear circuitry in the brain. This test includes a classical fear conditioning in that a stimulus (eg, light) is paired with a mild electric foot shock. During the fear-conditioning phase a light stimulus signals the occurrence of a shock. The startle response is elicited by a loud noise, and its amplitude is augmented when the light and the noise are presented together. BZs have anxiolytic effects in this paradigm in that they inhibit the enhancement of the startle response but do not block the startle response per se. Briefly, the paradigm involves placing the animal in a cage equipped to measure the amplitude of the startle response elicited by the noise, either in the presence or absence of a light previously paired with an electric shock. Animals that have already been exposed to the shock-paired light show a greater startle response to the noise in the presence of the light than in its absence. Using this kind of potentiated startle response as an operational measure, it was found that the central nucleus of the amygdala and a variety of hypothalamic and brain stem areas are involved in physiological (eg, activation of the sympathetic and the parasympathetic system, release of "stress hormones") and behavioral responses (eg, changes in locomotor activity, freezing) that reflect fear and anxiety.[54,55]

Defense tests

Defensive behaviors in mammals are thought to constitute a significant parameter that can be studied to understand human emotional disorders, including anxiety.[56] These behaviors occur in response to a number of threatening stimuli including predators, attacks by conspecifics, or presence of dangerous objects. The *mouse defense test battery* (MDTB) consists of an oval runway that allows the investigation of state anxiety by extensive ethological analyses to generate comprehensive behavioral profiles following drug treatment.[57,58] Specific situational and behavioral components of the *anxiety defense test battery*, including reac-

tivity to stimuli associated with potential threat such as presentation of an anesthetized predator (a rat), are incorporated into the MDTB. Drug experiments have demonstrated that anxiolytic compounds generally tend to decrease defensive behaviors. It is noteworthy that some responses are specifically or mainly modulated by certain classes of drugs, and it has been suggested that risk assessment, flight, defensive threat/attack and escape attempts probably reflect different aspects of anxiety-related reactions.[59] These tests may thus represent a considerable methodological improvement because a major concern with traditional animal models of state anxiety that are based on single measures is that they are often unable to discriminate between effects of different classes of anxiolytics (benzodiazepines, 5-HT_{1A} agonists, 5-HT reuptake inhibitors), whereas clinical findings strongly indicate differential therapeutic efficacy of these agents. Based on present observations in mice, the ethological plus-maze and the MDTB provide new tools to differentiate anxiolytic drugs of various classes that induce specific behavioral profiles.

Animal models for pathological anxiety

Pathological anxiety in humans is often an enduring feature of the individual, at least in part due to a genetic predisposition. To model genetically based anxiety, mice with target mutations in distinct genes were created that exhibit phenotypic changes indicative of increased anxiety. In addition rat or mouse lines were bred to select for high or low emotional reactivity.

The neurotransmitter 5-HT is centrally involved in the neuropathology of many neuropsychiatric disorders. More than a dozen pharmacologically distinct serotonin receptor subtypes regulate a wide range of functions in different brain areas and in the periphery of the body (for review, see ref 60). There is pharmacological and neuroanatomical evidence that at least one 5-HT receptor, 5-HT_{1A}, is involved in the regulation of anxiety-like behaviors.[49,61] Results of recent studies employing mutant mice with targeted deletions of the 5-HT_{1A} receptor gene further support a role of this receptor in anxiety.[62] 5-HT_{1A} receptor null mutant mouse lines have been independently generated in three laboratories from mice with different genetic backgrounds, C57BL/6,[63] 129/Sz,[62] or through outbreeding from Swiss-Webster.[64] Given the interlaboratory variability that occurred in other cases of behavioral studies on genetically modified mice,[65] it is notable that concordant findings on 5-HT_{1A} receptor null mutants were reported in all three laboratories and across the three mouse strains.

Further examples of models for pathological anxiety are mice that were gene targeted for the corticotropin-releasing factor (CRF)[66] or for the γ_2 subunit of the $GABA_A$ receptor. This receptor subunit is known to be essential in mediating the anxiolytic actions of benzodiazepines.[67] An "anxious" phenotype was also observed in mutant mice lacking the gene for the neuroactive peptide NPY [68]; (see also the review on genetic models of anxiety).[69]

At first glance, these lines of mutant mice seem to provide a unique opportunity to model pathological or trait anxiety. Moreover, compared with the state anxiety models in which the baseline level of anxiety of a subject is increased artificially by exposure to external (aversive) stimuli, the new models seem to be advantageous in that they may represent a kind of "general anxiety" due to a certain genetic modification. This sounds reasonable since genetic studies in humans have indicated that there are genetic components contributing to the development of anxiety disorders. However, one has to consider that in humans, the modulation of anxiety processes involves *multiple genes*. In the future, the use of mice strains that display elevated emotionality due to a distinct "genetic background," or mice selected for their high levels of anxiety using gene targeting experiments may lead to greater progress in our understanding of the neurobiological substrates of anxiety. Such animals would exhibit increased anxiety not because of a defect in a *single gene*, but because of a complex set of genes that result in an enduring feature of the strain/individual, thus determining its phenotype in combination with environmental factors.[46]

Inbred strains which show constantly high levels of anxiety/fearfulness have already been created. In mice, the BALB/c strain has been considered to be a realistic model of trait anxiety, which is probably not related to only one particular target gene but to abnormalities in various neurotransmitter circuits such as the GABAergic, dopaminergic and the opioid system.[46] Also in rats, several strains of trait anxiety have been described, eg, the Maudsley rat,[70] the Wistar–Kyoto,[71] the Roman,[72] or the Sardinian alcohol-preferring line.[73] Recently, two breeding lines were generated from the same strain of Wistar rats showing a maximum difference in anxiety-related behavior and a minimum difference in other behaviors as well as in physiological parameters not directly related to anxiety. These two rat lines are

now called *high anxiety-related behavior* (HAB) and *low anxiety-related behavior* (LAB).[74] Their overall performance in various behavioral tests suggests that selective breeding has resulted in lines not only differing markedly in their innate anxiety-related behavior but also in stress-related behavioral performances, indicating a close link between the emotional evaluation of a novel and stressful situation and a subject's capability to cope with such situations.

Developing novel models relevant to depression and anxiety disorders

One striking aspect of most anxiety disorders and MDD is the higher incidence in females compared with males.[9] Furthermore, gender differences in psychotropic drug metabolism and clearance can have direct effects on the efficacy of pharmacological treatments of mental disorders in women.[75] Thus, biological, hormonal, and cultural factors may contribute to gender differences in some disorders and to gender-specific efficacy of pharmacological interventions. Basic research in animals may help to determine the degree to which these features are caused by differences in brain physiology.[76] Given the preponderance of sex differences in many aspects of anxiety disorders and MDD, it is surprising to find how few basic animal studies have considered gender as a determining factor for depression and anxiety disorders. A recent survey revealed that approximately 90% of the animal studies on serotonergic drugs and anxiety-like behaviors utilized males exclusively.[77] Clearly, this major deficiency has delayed progress towards an understanding of the processes contributing to anxiety disorders and MDD, and most likely hindered the development of gender-specific treatments.

Conclusion

In conclusion, animal models are indispensable tools for research on the neurobiological mechanisms underlying MDD and/or anxiety disorders and for the development of new antidepressant/anxiolytic drugs. However, besides the obvious progress in research that could only be achieved because of the existence of these models, one also has to bear in mind that each animal model has its pros and cons. Currently, it appears that the use of several models, either successively or in parallel, provides the greatest chance to elucidate the neurobiological processes of psychiatric diseases and to identify new, effective antidepressant and anxiolytic compounds. ❑

REFERENCES

1. McKinney WT. Animal models of depression: an overview. *Psychiatr Dev.* 1984;2:77-96.
2. Willner P. *Behavioural Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives*. Cambridge, UK: Cambridge University Press; 1991.
3. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci.* 2002;23:238-245.
4. Mineka S, Watson D, Clark LA. Comorbidity of anxiety and unipolar mood disorders. *Annu Rev Psychol*. 1998;49:377–412.
5. Kaufman J, Charney D. Comorbidity of mood and anxiety disorders. *Depress Anxiety*. 2000;12 (suppl I): 69-76.
6. Nemeroff C. Comorbidity of mood and anxiety disorders: the rule, not the exception. *Am J Psychiatry*. 2002;159:3-4.
7. Gorman JM. Comorbid depression and anxiety spectrum disorders. *Depress Anxiety*. 1997;4:160-168.
8. Nelson JC. A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol Psychiatry*. 1999;46:1301-1308.
9. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. Washington, DC: American Psychiatric Association; 1994.
10. Jesberger JA, Richardson JS. Animal models depression: parallels and correlates to serve depression in humans. *Biol Psychiatry*. 1985;20:764-784.
11. Sanchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol*. 2001;13:419-449.
12. Seligman M, Maier S. Failure to escape traumatic shock. *J Exp Psychol*. 1967;74:1-9.
13. Nestler EJ, Gould E, Manji H, et al. Preclinical models: status of basic research in depression. *Biol Psychiatry*. 2002;52:503-528.
14. O'Neil MFO, Moore NA. Animal models of depression: Are there any? *Human Psychopharmacol*. 2003;18:239-254.
15. Vollmayr B, Henn FA. Learned helplessness in the rat: improvements in validity and reliability. *Brain Res Protocols*. 2001;8:1-7.
16. Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neurosci Biobehav Rev*. 1992;16:525-534.
17. Anisman H, Zacharko RM. Depression the predisposing influence of stress. *Behav Brain Sci*. 1982;5:89-137.
18. Kessler RC. The effects of stressful life events on depression. *Annu Rev Psychol*. 1997;48:191-214.
19. Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry*. 1999;156:837-841.
20. Paykel ES. Stress and affective disorders in humans. *Semin Clin Neuropsychiatry*. 2001;6:4-11.
21. Yadid G, Nakash R, Deri I, et al. Elucidation of the neurobiology of depression: insights from a novel genetic animal model. *Prog Neurobiol*. 2000;62:353-378.
22. Brown G. Life events and illness. In: Stanford SC, Salamon P, eds. *Stress: from Synapse to Syndrome*. London, UK: Academic Press; 1993:20-40.
23. Blanchard DC, Blanchard RJ. Behavior correlates of chronic dominance-subordination relationships of adult male rats in a seminatural situation. *Neurosci Biobehav Rev*. 1990;14:455-462.
24. Fuchs E, Flügge G. Social stress in tree shrews: effects on physiology, brain function and behavior of subordinate individuals. *Pharmacol Biochem Behav*. 2002;73:247-258.

Modelos experimentales de animales para la simulación de depresión y ansiedad

Hoy en día existe un gran número de modelos animales para evaluar la depresión y la ansiedad. Sin embargo, la relación entre estos modelos y los síndromes clínicos de depresión y ansiedad no siempre es clara. Ya que los trastornos de ansiedad en humanos representan un fenómeno multifactorial frecuentemente comórbido con la depresión mayor o/y otros problemas psiquiátricos, la posibilidad de crear modelos animales que reflejen consistentemente la situación en humanos es baja. Cuando se utilizan modelos animales para intentar comprender las semejanzas entre la conducta animal y humana, se debe considerar el contexto en el cual un animal está siendo investigado, y el significado funcional y la importancia de los parámetros conductuales que son cuantificados. Además, las variabilidades de género, interindividuales e interespecies en las respuestas conductuales a la situación de prueba y en la sensibilidad a los tratamientos farmacológicos son fuentes potenciales de resultados desconcertantes. En el pasado, estos aspectos han sido a menudo descuidados en las aproximaciones preclínicas a la farmacología conductual y a la psicofarmacología. Para imitar uno o más aspectos relevantes de los trastornos de ansiedad patológica y de la depresión se requiere de una aproximación pragmática que combine esfuerzos preclínicos y clínicos. Los modelos resultantes pueden identificar procesos del sistema nervioso central que regulan la producción de una conducta definida, con la posibilidad de desarrollar tratamientos más efectivos.

Modèles animaux expérimentaux pour simuler la dépression et l'anxiété

Il existe aujourd'hui un grand nombre de modèles animaux pour évaluer la dépression et l'anxiété. Les relations entre ces modèles et les syndromes cliniques de dépression et d'anxiété ne sont cependant pas toujours claires. La possibilité de créer des modèles animaux imitant avec validité les troubles anxieux est assez faible, puisque ces troubles sont multifactoriels, se présentant fréquemment en comorbidité avec un état dépressif majeure et/ou d'autres problèmes psychiatriques. Il y a lieu de prendre en considération le contexte d'observation de l'animal, ainsi que le sens et la validité des comportements étudiés quantitativement, lors de l'utilisation de modèles expérimentaux afin de comprendre les points communs entre les comportements animaux et humains. De plus, le sexe et les différences interindividuelles et interespèces au niveau des réponses comportementales aux situations des tests ainsi qu'à la sensibilité aux traitements pharmacologiques sont une source potentielle d'erreur. Dans le passé ces aspects ont souvent été négligés lors des études précliniques en pharmacologie du comportement et en psychopharmacologie. Une approche pragmatique combinant les thèmes précliniques et cliniques est nécessaire pour modéliser un ou plusieurs aspects pertinents de la dépression et de l'anxiété. Les modèles résultant de cette approche peuvent permettre l'identification de processus cérébraux gérant les conséquences comportementales, donc ayant un potentiel pour le développement de médicaments plus efficaces.

25. Rygula R, Abumaria N, Flugge G, et al. Anhedonia and motivational deficits in rats: impact of chronic social stress. *Behav Brain Res*. 2005;162: 127-134.
26. Miczek KA. Tolerance to the analgesic, but not discriminative stimulus effects of morphine after brief social defeat in rats. *Psychopharmacology (Berl)*. 1991;104:181-186.
27. Koolhaas JM, De Boer SF, De Rutter AJ, et al. Social stress in rats and mice. *Acta Physiol Scand Suppl*. 1997;640:69-72.
28. Rygula R, Abumaria N, Flugge G, et al. Citalopram counteracts depressive-like symptoms evoked by chronic social stress in rats. *Behav Pharmacol*. 2006;17:19-29.
29. Abumaria N, Rygula R, Havemann-Reinecke U, Ruther E, Bodemer W, Roos C, Flugge G. Identification of genes regulated by chronic social stress in the rat dorsal raphe nucleus. *Cell Mol Neurobiol*. 2006 Apr 22 [Epub ahead of print].
30. Duman RS. Neuroplasticity: consequences of stressand actions of antidepressant treatment. *Dialogues Clin Neurosci*. 2004;6:157-169.
31. Willner P, Mitchell PJ. Animal models of depression: a diathesis/stress approach. In: D'haenen HAH, Den Boer JA, Willner P, eds. *Biological Psychiatry*. Chichester, UK: Wiley; 2002:703-726.
32. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry*. 2000;157:1552-1562.
33. Frazer A, Morilak DA. What should animal models of depression model? *Neurosci Biobehav Rev*. 2005;29:515-523.
34. Mitchell PJ, Redfern PH. Animal models of depressive illness: the importance of chronic drug treatment. *Curr Pharmaceutical Design*. 2005;11:171-203.
35. Matthews K, Christmas D, Swan J, et al. Animal models of depression: navigating through the clinical fog. *Neurosci Biobehav Rev*. 2005;29:503-513.
36. Martignoni E, Petraglia F, Costa A, et al. Dementia of the Alzheimer type and hypothalamus–pituitary–adrenocortical axis: changes in cerebrospinal fluid corticotropin releasing factor and plasma cortisol levels. *Acta Neurol Scand.* 1990;81:452-456.

37. Contoreggi C, Herning RI, Na P, et al. Stress hormone responses to corticotropin-releasing hormone in substance abusers without severe comorbid psychiatric disease. *Biol Psychiatry*. 2003;54: 873-878.
38. Strickland PL, Deakin JFW, Percival C, et al. Bio-social origins of depression in the community: interactions between social adversity, cortisol and serotonin neurotransmission. *Br J Psychiatry*. 2002;180:168-173.
39. Shekhar A, McCann UD, Meany MJ, et al. Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. *Psychopharmacology*. 2001;157:327-339.
40. Rodgers RJ. Animal models of 'anxiety': where next? *Behav Pharmacol*. 1997;8:477-496.
41. Goa KL, Ward A. Buspirone: a preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. *Drugs*. 1986;32:114-129.
42. Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*. 1987;92:180-185.
43. Lister RG. Ethologically-based animal models of anxiety disorders. *Pharmacol Ther*. 1990;46:321-340.
44. Treit D. Animal models for the study of anti-anxiety agents: a review. *Neurosci Biobehav Rev*. 1985;9:203-222.
45. Sanger DJ. Animal models of anxiety and the screening and development of novel anxiolytic drugs. In: Boulton AA, Baker GB, Martin-Iverson MT, eds. *Animal Models in Psychiatry II, Neuromethods*. Vol 19. Clifton, NJ: Humana Press; 1991:147-198.
46. Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res*. 2001;125:141-140.
47. Griebel G. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther*. 1995;65:319-395.
48. Rodgers RJ, Cao BJ, Dalvi A, et al. A. Animal models of anxiety: an ethological perspective. *Braz J Med Biol Res*. 1997;30:289-304.
49. Weiss SM, Lightowler S, Stanhope KJ, Kennett GA, Dourish CT. Measurement of anxiety in transgenic mice. *Rev Neurosci*. 2000;11:59-74.
50. Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol Biochem Behav*. 1996;54:21-30.
51. Rodgers RJ, Johnson NJT. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav*. 1995;52:297-303.
52. Kelley AE. Locomotor activity and exploration. In: Sahgal A, ed. *Behavioural Neuroscience. A Practical Approach*. Vol II. Oxford, UK: IRL Press; 1993. 1-21.
53. File SE. Animal models for predicting clinical efficacy of anxiolytics: social behaviour. *Neuropsychobiology*. 1985;13:55-62.
54. Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends Pharmacol Sci*. 1992;13:35-41.
55. Davis M, Falls WA, Campeau S, Kim M. Fear-potentiated startle: a neural and pharmacological analysis. *Behav Brain Res*. 1993;58:175-198.
56. Blanchard RJ, Blanchard DC. Affect and aggression: an animal model applied to human behavior. In: Blanchard RJ, Blanchard DC, eds. *Advances in the Study of Aggression*. Orlando, Fla: Academic Press Inc; 1984:1-62.
57. Griebel G, Blanchard DC, Jung A, Blanchard RJ. A model of 'antipredator' defense in Swiss-Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities. *Behav Pharmacol*. 1995;6:732-745.
58. Blanchard DC, Griebel G, Blanchard RJ. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. *Neurosci Biobehav Rev*. 2001;25: 205-281.
59. Griebel G, Sanger DJ. The mouse defense test battery: an experimental model of different emotional states. In: Haug M, Whalen RE, eds. *Animal Models of Human Emotion and Cognition*. Washington, DC: American Psychological Association; 1999:75-85.
60. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology*. 1999;38:1083-1152.
61. File SE, Gonzalez LE, Andrews N. Comparative study of pre- and postsynaptic 5HT1A receptor modulation of anxiety in two ethological animal tests. *J Neurosci*. 1996;16:4810-4815.
62. Ramboz S, Oosting R, Amara DA, et al. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A*. 1998;95:14476-14481.
63. Heisler LK, Chu HM, Brennan TJ, et al. Elevated anxiety and antidepressant-like responses in serotonin 5-HT 1A receptor mutant mice. *Proc Natl Acad Sci U S A.* 1998;95:15049-15054.
64. Parks CL, Robinson PS, Sibille E, et al. Increased anxiety of mice lacking the serotonin 1A receptor. *Proc Natl Acad Sci U S A.* 1998;95:10734-10739.
65. Crabbe JC, Wahlsten D, Dudek BC. Genetics of mouse behavior: interactions with laboratory environment. *Science*. 1999;284:1670-1672.
66. Stenzel-Poore MP, Duncan JE, Rittenberg MB, et al. CRH overproduction in transgenic mice: behavioral and immune system modulation. *Ann NY Acad Sci.* 1996;780:36-48.
67. Crestani F, Lorez M, Baer K, et al. Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat Neurosci*. 1999;2:833-839.
68. Bannon AW, Seda J, Carmouche M, et al. Behavioral characterization of neuropeptide Y knockout mice. *Brain Res*. 2000;868:79-87.
69. Finn DA, Rutledge-Gormann MT, Crabbe JC. Genetic animal models of anxiety. *Neurogenetics*. 2003;4:109-135.
70. Broadhurst PL. The Maudsley reactive and non-reactive strains of rats: a survey. *Behav Genet*. 1075;5: 299-319.
71. Goto SH, Conceicao IM, Ribeiro RA, Frussa Filho R. Comparison of anxiety measured in the elevated plus-maze, open-field and social interaction tests between spontaneously hypertensive rats and Wistar EPM-1 rats. *Braz J Med Biol Res*. 1993;26:965- 969.
72. Chaouloff F, Castanon N, Mormede P. Paradoxical differences in animal models of anxiety among the Roman rat lines. *Neurosci Lett*. 1994;182:217-221.
73. Colombo G, Agabio R, Lobina C, et al. Sardinian alcohol-preferring rats: a genetic animal model of anxiety. *Physiol Behav*. 1995;57:1181-1185.
74. Liebsch G, Montkowski A, Holsboer F, et al. Behavioural profiles of two Wistar lines selectively bred for high or low anxiety-related behaviour. *Behav Brain Res*. 1998;94: 301-310.
75. Pigott TA. Gender differences in the epidemiology and treatment of anxiety disorders. *J Clin Psychiatry*. 1999;60:4-15.
76. Palanza P. Animal models of anxiety and depression: how are females different? *Neurosci Biobehav Rev*. 2001;25:219-233.
77. Blanchard DC, Griebel G, Blanchard RJ. Gender bias in preclinical psychopharmacology: male models for (predominantly) female disorders. *J Psychopharmacol*. 1995;9:79-82.

The role of serendipity in drug discovery

Thomas A. Ban, MD, FRCP(C)

Serendipity is one of the many factors that may contribute to drug discovery. It has played a role in the discovery of prototype psychotropic drugs that led to modern pharmacological treatment in psychiatry. It has also played a role in the discovery of several drugs that have had an impact on the development of psychiatry. "Serendipity" in drug discovery implies the finding of one thing while looking for something else. This was the case in six of the twelve serendipitous discoveries reviewed in this paper, ie, aniline purple, penicillin, lysergic acid diethylamide, meprobamate, chlorpromazine, and imipramine. In the case of three drugs, ie, potassium bromide, chloral hydrate, and lithium, the discovery was serendipitous because an utterly false rationale led to correct empirical results; and in case of two others, ie, iproniazid and sildenafil, because valuable indications were found for these drugs which were not initially those sought. The discovery of one of the twelve drugs, chlordiazepoxide, was sheer luck.

 Dialogues Clin Neurosci. 2006;8:335-344.

Keywords: *chloral hydrate; chlorpromazine; imipramine; iproniazid; lithium; lysergic acid diethylamide; meprobamate; penicillin; serendipity; sildenafil*

Author affiliations: Emeritus Professor of Psychiatry, Vanderbilt University, Nashville, Tenn, USA

Address for correspondence: Prof Thomas A. Ban, 1177 Yonge Street, Suite 607, Toronto, Ontario, Canada M4T 2Y4
(e-mail: fmcp@allstream.net)

Definition of serendipity

Serendip is the old Arabic name for Ceylon, now known as Sri Lanka. The origin of the word "serendipity" is in a Persian fairy tale, The Three Princes of Serendip, whose traveling heroes were "always making discoveries, by accidents and sagacity, of things they were not in quest of."[1] In the 16th century, the tale was translated from Persian to Italian, and from Italian to French. Horace Walpole (1717-1797), an English man of letters, encountered it in a collection of oriental tales in French, and coined the English term "serendipity" in a letter to his friend, Horace Mann, dated June 28, 1754.[2]

Today, the word "serendipity" is a word that is used in everyday language. The *Oxford English Dictionary* defines it as "the faculty of making happy and unexpected discoveries by accident," and *Webster's New Collegiate Dictionary* as "the faculty of finding valuable or agreeable things not sought for."[3] In *Stedman's Medical Dictionary* "serendipity" refers to "an accidental discovery;" ie, "finding one thing while looking for something else."[4]

According to the Doctor Out of Zebulon column in the *Archives of Internal Medicine*, "serendipity signifies a mental state in which serenity and stupidity are blended," as for example, "the serendipity of a cow chewing its cud under a shady tree," or "the sort of thing that happens to you when on a dull day collecting fossils you find instead a beautiful woman who proves to be neither geologist nor archeologist."[5,6] However, this definition is erroneous, at least insofar as scientific discoveries are concerned. No scientific discovery has ever been made by pure luck. All happy accidents in science have one point in common: "each was recognized, evaluated and acted upon in the

Selected abbreviations and acronyms

5-HT	*serotonin*
CPZ	*chlorpromazine*
LSD	*lysergic acid diethylamide*
MAO	*monoamine oxidase*
NO	*nitric oxide*

light of the discoverer's total intellectual experience."[7] "Chance favors the prepared mind," as Pasteur (1822-1896) said, or more precisely: "Dans les champs de l'observation, le hasard ne favorise que les esprits préparés."[8] Indeed, it is hard to think of a better expression of "serendipity" as one reviews the incredible concatenation of intentional and chance events in medicine's happy accidents.[2,9]

Development of the drug industry

The story begins in 1856 with an 18-year-old English chemist named William Henry Perkins (1838-1907) who was trying to synthesize quinine and ended up with a bluish substance, that he extracted from a "black mess" in his test tube, which had excellent dyeing properties.[10] Perkins' discovery of the first artificial dye in history, variably referred to as aniline purple, tyrian blue, or mauve, triggered a chain reaction by serendipity.[7] Modifications of his process led to the development of many dyes and the emergence of the dye industry, eg, Bayer (1862), Ciba (1859), Geigy (1859), and Sandoz (1862).[10,11] Recognition that a fuller exploitation of his findings would require a new breed of chemist[12] gave a strong impetus for the development of organic chemistry.[13,14] The synthesis of organic compounds led to the birth of the pharmaceutical industry.[15] By the end of the 19th century, many of the dye companies, eg, Bayer (1896) and Ciba (1889),[12] extended their activities to the development of drugs.

Perkins' discovery cannot be attributed to pure luck. He studied at the Royal College of Chemistry in London under August Wilhelm von Hofmann (1818-1892), one of the pioneers of aniline chemistry,[16] and was aware that crystalline (a substance obtained by O. Unverdorben in 1826 by distillation of indigo) and kyanol or cyanol (a substance isolated from coal tar by F. Runge in 1834, that produced a beautiful blue color on treatment with calcium chloride), were the same substance (phenylamine, with the composition of $C_5H_5NH_2$) that C. J. Fritzsche obtained by treating indigo with potassium chloride, and named aniline. (The word "aniline" comes from *Indigofera anil*, the indigo-yielding plant; anil is derived from the Sanskrit word "nile," ie, dark blue.[17]) His serendipitous discovery was built on his knowledge and past experience. He was also fully aware of the potential use of his discovery.

Early drugs in psychiatry

The introduction of the first effective drugs for the control of excitement, agitation, and insomnia paralleled the birth of the pharmaceutical industry. In the clinical development of at least two of these drugs, potassium bromide and chloral hydrate, serendipity played an important role.

Potassium bromide

Potassium bromide is the oldest widely used sedative in medicine. It is the potassium salt of bromine, a chemical element, first isolated in 1826 from the ashes of seaweed by A. J. Balard, an apothecary in Montpelier, France.[18] In its natural form bromine is too corrosive to be ingested. As a potassium salt it is well tolerated.[19]

French clinicians believed that bromine was a substitute for iodine, and began using potassium bromide in a variety of disorders without tangible therapeutic effect. In 1857, 31 years after bromine was isolated, Charles Lockock, a London internist, discovered the anticonvulsant and sedative action of the drug.[20] His discovery was one of the many quaint examples of serendipity in which an utterly false theory led to correct empirical results. Lockock, like most physicians of his time, believed that there was a cause-effect relationship between masturbation, convulsions, and epilepsy. Bromides were known to curb the sex drive. Lockock's rationale was to control epilepsy, ie, convulsions, by reducing the frequency of masturbation.[21] The treatment was a success insofar as control of convulsions was concerned. It also brought to attention the sedating properties of the drug.

During the second half of the 19th century, potassium bromide and other inorganic bromide salts were widely used as anxiolytic sedatives and anticonvulsants.[22] They were undoubtedly effective, although their relatively low therapeutic efficacy coupled with high toxicity have today all but eliminated them from clinical use.[23]

Chloral hydrate

Similar to potassium bromide, the discovery of the sedative and hypnotic properties of chloral hydrate was also

the result of an erroneous idea, but in this case of a chemical theory.
Chloral, or trichloroacetaldehyde, was first prepared in 1832 by Justus von Liebig, a professor of chemistry in Giessen (Germany).[24] It was about 37 years later, in 1869, that its hydrate, chloral hydrate, was introduced into clinical therapeutics by Otto Liebreich, a professor of pharmacology in Berlin.[25] Liebreich assumed that one of the components into which chloral hydrate splits in the body is chloroform, and since chloroform induces sleep, so would chloral hydrate. Although no chloroform resulted from the degradation of chloral hydrate, chloral hydrate became the first synthetically produced reliable hypnotic; today, after almost 140 years, it is still used in clinical practice.[17]

Lithium in mood disorders

This discovery and rediscovery of the therapeutic effects of lithium in psychiatry were the result of false theories about the etiology of mood disorders.

Discovery in the 1880s

Lithium is an alkali metal that was discovered by J. A. Arfvedson in 1817 while analyzing the mineral petalite. The name lithium comes from the Greek "lithos," stone; it was coined by Jons Jacob Berzelius (1779-1848), who was involved in classifying minerals.[26] The substance was first isolated in sufficient quantity for medical use by R. Bunssen and A. Mathiessen, in 1855. Four years later, after the demonstration that lithium carbonate could dissolve urate stones,[27] the substance was introduced into medicine for the treatment of gout by Alfred Barring Garrod.[28] Gout is a disease with urate deposits in the cartilage and increased uric acid, a breakdown product of urea, in the blood.
During the second part of the 19th century, many physicians believed in a uric acid "diathesis," a predisposition for the accumulation of urea in the body,[29] that could cause a variety of disorders from gout and rheumatism to cardiac disease and mental illness.[27] Since acute symptoms of gout develop suddenly and persist untreated for days or weeks before they remit, William Hammond, at the Bellevue Hospital in New York, had assumed that mood disorders might be a form of cerebral gout and employed lithium successfully in their treatment.[30,31] On the basis of the same assumption, Carl Lange, a Danish neurologist, treated hundreds of patients with lithium and reported on its prophylactic effect in periodic mood disorders in 1896.[32] Yet, without the availability of the necessary technology for monitoring blood levels, lithium was too toxic a substance to be clinically employed.

Rediscovery in the 1940s

In the late 1940s the therapeutic effect of lithium in mania was rediscovered by John Cade, an Australian psychiatrist.[33] Operating on the assumption that manic-depressive illness is analogous to thyrotoxicosis and myxedema, he hypothesized that mania is a state of intoxication by a normal product of the body in excess, and melancholia is a state of deficiency of the same substance. To test this hypothesis he compared the effects of intraperitoneally injected concentrated urine from manic subjects with urine from normal subjects in guinea pigs, and found the former far more toxic in killing the animals than the latter. Cade identified urea as the culprit that killed the animals, and established that creatinine decreased ("protected") whereas uric acid increased ("enhanced") the toxicity of urine. Since the urine of manic patients was more toxic than could be neutralized by the protective action of creatinine, he decided to determine the toxicity-enhancing effect of uric acid. Because uric acid was virtually insoluble in water, he used the most soluble of the urates, lithium urate, in his experiments. To his surprise, instead of enhancing toxicity, lithium urate protected the animals from urea's toxic effects. He attributed the protective effect to lithium, and demonstrated that injection of an 8% urea solution killed five of 10 guinea pigs, whereas a similar solution with lithium added killed none.[34]
To determine whether lithium salts alone have any discernable effects, Cade injected large doses of 0.5% aqueous solution of lithium carbonate into guinea pigs, and found that after a latent period the animals became extremely lethargic and unresponsive to stimuli for about 2 hours. It may seem a long way from the lethargy of guinea pigs to the control of manic excitement, but since Cade's investigations had commenced in an attempt to demonstrate the presence of a toxic substance excreted in the urine of manic patients, he decided to compare the effect of lithium in ten manic, six schizophrenic, and five depressed patients. The substance was effective in controlling psychotic excitement, especially in manic patients.[33]

Cade's rediscovery of the therapeutic effect of lithium in mania led to systematic clinical investigations with the substance in the 1950s by Mogens Schou and his associates in Denmark, verifying the therapeutic effect of lithium in mania,[35] and rediscovering in the 1960s its prophylactic effect in manic-depressive psychosis and recurrent depression.[36] Since by the 1960s the substance could be safely administered, with the employment of the flame spectrophotometer for monitoring blood levels, lithium has remained the primary form of treatment in manic-depressive illness, referred to as bipolar disorder in current consensus-based classifications.[37]

The story of LSD-25

Cade's notion that mania is the manifestation of a toxic agent was in keeping with contemporary thinking about the biology of psychoses. One of the strong influences on the Zeitgeist was Rolv Gjessing's discovery in the mid-1930s of nitrogen retention in certain phases of periodic catatonia,[38] and his postulation that altered metabolism with the production of a mescaline-like substance was responsible for catatonia.[39] Another influence on the Zeitgeist was Swiss chemist Albert Hofmann's discovery of the psychotomimetic effect of lysergic acid diethylamide (LSD-25), a synthetic amide of the ergot alkaloid, lysergic acid,[40] in the early 1940s.

Ergot is a biological product of a growing fungus, *Claviceps purpura*, which had been used by women for inducing contractions of the uterus since the Middle Ages. It was introduced into medicine as a uterotonic by an American physician John Stearns in 1808.[41] Lysergic acid was first isolated from ergot by alkaline hydrolysis in 1933 by Jacobs and Craig.[42] In the late 1930s a new procedure was developed that allowed combining lysergic acid with amides in peptide linkage. It led to the first partial synthesis of a natural ergot alkaloid, ergometrine, a uterotonic, and, by modifying the alkanolamine side chain of ergometrine, to a synthetic ergot derivative, methergine, a hemostatic. In 1938, Hofmann, working in the laboratories of Sandoz, prepared lysergic acid diethylamide, a substance structurally related to the circulatory stimulant nikethamide, with the objective of developing an analeptic. Since the substance was the 25th compound of the lysergic acid amide series, it was given the code name LSD-25.[43] In pharmacological testing LSD-25 produced uterine contraction, similar to that of ergometrine. Excitation was observed in some animals after LSD-25 administration. The findings did not warrant immediate further exploration.

On April 16, 1943, while preparing a new supply of LSD-25, Hofmann was struck by a strange feeling that made him stop work in the mid-afternoon. He reported the following to his superior:

> …I was seized by a peculiar restlessness associated with a sensation of mild dizziness. On arriving home I lay down and sunk into a kind of drunkenness which was not unpleasant and which was characterized by extreme activity and imagination. As I lay in a dazed condition with my eyes closed (I experienced daylight as disagreeably bright) there surged upon me an uninterrupted stream of fantastic images of extraordinary plasticity and vividness and accompanied by the intense, kaleidoscopic play of colors. The condition gradually passed off after about two hours.[43]

Hofmann suspected that LSD-25 was the culprit, but could not figure how the substance "found its way into his body in sufficient quantity to produce such extraordinary phenomena." Moreover, the nature of his symptoms did not correspond with those previously reported with ergot poisoning. To get to the "root of the matter" he decided to conduct experiments with LSD-25 on himself. Since he took relatively high doses of the substance, the psychotomimetic effects were even more pronounced than on the first occasion.[43]

Although the discovery of the psychotomimetic effect of LSD-25 is usually attributed to serendipity, Hofmann maintains that "LSD was not the fruit of a chance discovery, but the outcome of a more complex process that had its beginnings in a definite concept, and was followed up by appropriate experiments, during the course of which a chance observation served to trigger a planned investigation, which then led to the actual discovery."[43] He was also aware that the discovery of the psychotomimetic effect of LSD "lent support to the hypothesis that certain mental illnesses that were supposed until then to be purely psychic in nature had a biochemical cause because it now seemed feasible that undetectable traces of a psychoactive substance produced by the body itself might produce psychic symptoms."[43]

In the mid-1940s, demonstration of the therapeutic effect of penicillin in primary syphilis and neurosyphilis with its implications for psychiatry distracted attention from Hofmann's discovery. It was more than 10 years later in the early 1950s that interest in LSD was revived after Woolley and Shaw's demonstration that it inhibited the neurotransmitter serotonin.[43] LSD became instrumental

also to the revival of experimental psychiatry in the mid-1950s because it is reasonable to assume, as Mayer-Gross pointed out, that psychological symptoms that can be provoked by a drug, can also be abolished by drug action.[39]"

Discovery of penicillin

The serendipitous discovery of penicillin in 1928 by Alexander Fleming led to major changes in the diagnostic distribution of psychiatric patients in the late 1940s. Fleming was engaged in research on influenza when one of his staphylococcus culture plates had become contaminated and developed a mold that created a bacteria-free circle.[44] Since he was working in an old building with considerable dust, where contamination was likely to occur, many bacteriologists would not have thought it particularly remarkable that one particular colony of staphylococci was undergoing dissolution, for it has long been known that some bacteria interfere with the growth of others. However, Fleming recognized the possible significance of the bacteria-free circle,[45] and by isolating the mold in pure culture he found that it produced a substance that has a powerful destructive effect on many of the common bacteria that infect man. He named the antibacterial substance liberated into the fluid in which the mold was grown "penicillin," after *Penicillium notatum*, the contaminant of the staphylococcus colony that led to the dicovery.[46]

Although Fleming published his results in the *Journal of Experimental Pathology* in 1929,[44] it was only 10 years later that Howard Florey and his team embarked on the research that culminated in 1941 in the development of a methodology for the extraction and production of penicillin. To obtain sufficient quantity of the substance for clinical use, the original strain, *Penicillium notatum*, had to be replaced by *Penicillium chrysogenum*.[45] Two years later, John Mahoney and his associates in the US Public Health Service, demonstrated that penicillin was highly effective in the treatment of primary syphilis;[20] and in 1944, Stokes and his associates at Johns Hopkins Hospital in Baltimore, Maryland, reported on the therapeutic effect of penicillin in the treatment of "late syphilis including neurosyphilis."[47] Since neurosyphilis and infectious delirium represented a considerable proportion of psychiatric patients, by changing the diagnostic distribution of patients, the introduction of penicillin resulted in a shift in priorities in psychiatric research from the "organic" to the "functional" psychiatric disorders by the end of the 1940s.[17]

Anxiolytic drugs

The introduction of penicillin stimulated the industry to develop other antibiotics. The development of meprobamate, the first anxiolytic drug introduced into clinical practice, was the result of a serendipitous observation in the course of this research.

Meprobamate

Research that led to the development of meprobamate began in 1945 in the laboratories of the British Drug Houses Ltd (BDH) in London. Chemists were to develop nontoxic antibacterial agents that would inhibit the growth of Gram-negative micro-organisms that cause enzymatic destruction of penicillin. Since the only compound known at the time that had properties of this type was phenoxetol, the phenyl ethyl ether of phenol, Frank Berger examined several structurally related α-substituted ethers of glycerol—synthesized by William Bradley, the chief chemist of BDH—for their antibacterial and pharmacological action.[48] It was in the course of this research that Berger noted that "administration of small quantities of α-substituted ethers to mice, rats, or guinea pigs caused *tranquilization*, muscular relaxation, and a sleep-like condition from which the animal could be roused."[49] Impressed with the tranquilization and muscle relaxation produced by these drugs, Berger pursued his further research with mephenesin, or 3-(2-methoxyphenyl)-1,2-propanediol, the substance from the series that possessed the most intense muscle relaxant action and widest margin of safety.[50] Mephenesin was an old drug; it was first produced by the condensation of o-cresol with glycerine by Zivkovic in 1908.

Berger moved to the United States in 1948, and in the same year mephenesin was released for clinical use for muscular relaxation during light anesthesia, under the trade name Tolserol by E. R. Squibb. The drug was already in clinical use when it was recognized that it could relieve anxiety and tension. However, mephenesin had serious drawbacks, eg, short duration of action and greater effect on the spinal cord than on supraspinal structures. To overcome these disadvantages, Berger succeeded in initiating a program that yielded the synthesis of meprobamate, or 2-methyl-2-n-propyl-1,3-propanediol dicarbamate, by B. J. Ludwig, at the Wallace Laboratories of Carter Products, in May 1950.[48,51] The duration of action of the new drug was about eight times

longer than that of the parent substance. Similar to mephenesin, pharmacologically meprobamate was a tranquilizer. It depressed multineuronal reflexes without significantly affecting monosynaptic reflexes; counteracted pentylenetetrazol–induced convulsions, and produced a loss of the righting reflex in mice without causing significant excitement prior to the onset of the paralysis. In the spring of 1955 Lowell Selling was first to report on the therapeutic effect of meprobamate in anxiety and tension states. A few months later, in the summer of 1955, meprobamate was introduced into clinical use by Wallace Laboratories with the brand name of Miltown, the name of the small community in New Jersey where Berger lived at the time,[52] and by Wyeth Laboratories with the brand name of Equanil.[51]
By the late 1950s meprobamate was the most widely used prescription drug in the United States and in many other countries. It retained its lead until the late 1960s when it succumbed to diazepam, the second drug from the benzodiazepine series introduced into clinical use.[48,53]

Chlordiazepoxide

The synthesis of benzodiazepines is linked to the name of Leo Sternbach, a pharmacist and chemist working at Hoffmann-La Roche's research facility at Nutley, New Jersey (USA).
In the early 1930s Sternbach was a postgraduate student at the Jagellonian University in Cracow, Poland, and synthesized several heptoxdiazine compounds in an effort to develop synthetic dyes. In 1954, inspired by the phenomenal success of chlorpromazine and early reports on meprobamate, he resumed his research with heptoxdiazines with the hope of finding compounds with psychopharmacological activity.[54] In the course of this research he recognized that the drugs he perceived in the 1930s as heptoxdiazines were benzoxadiazepines, and synthesized about 40 benzoxadiazepine compounds. Although all of the newly synthesized drugs that were tested were pharmacologically inert, Sternbach decided to stabilize one of the benzoxadiazepines with methylamine, a primary amine, instead of using secondary or tertiary amines as in the pharmacologically inert derivatives. He labeled the stabilized compound Ro 5-0690, and placed it on the shelf. In 1957, Ro 5- 0690 was found, literally during a laboratory cleanup, and submitted for pharmacological evaluation, which showed that it had similar activities to meprobamate. This was sheer luck! Prompted by these findings, the structure of Ro 5-0690 was correctly identified as 1,4-benzodiazepine.
Ro 5-0690, the first anxiolytic benzodiazepine, was introduced into clinical use in 1960 with the generic name of methaminodiazepoxide (chlordiazepoxide), and the brand name of Librium. It was followed by the introduction of diazepam (Valium), another anxiolytic benzodiazepine, in 1963. From the late 1960s through the 1970s, sales of diazepam topped those of all other drugs in the United States.
The introduction of benzodiazepines vastly extended the use of psychotropic drugs, ranging from the treatment of schizophrenia, depression, and bipolar disorder to the alleviation of anxiety and other neurotic conditions, making psychotropic drugs one of the most prosperous businesses of the pharmaceutical industry.

Psychotropic drugs

The term "psychotropic" was coined by Ralph Gerard, an American neurophysiologist, in the mid-1950s,[17] for drugs with an effect on mental activity and behavior. During the 1950s, a series of new psychotropic drugs, such as chlorpromazine, imipramine, and iproniazid, were introduced. Their effectiveness in the treatment of schizophrenia, depression, and bipolar disorder was instrumental in shifting the site of psychiatric practice from psychiatric hospitals to the community.

Chlorpromazine

Chlorpromazine (CPZ), has a phemothiazine nucleus with a dimethylaminopropyl side chain. Synthesized by Paul Charpentier on December 11, 1950, in the Laboratories of Rhône Poulenc, at the time a major French pharmaceutical company, CPZ was released in May 1951 for clinical investigation as a potentiator of general anesthesia.[55]
The basic phenothiazine nucleus was synthesized by Bernthsen in 1883, and later introduced as an anthelminthic agent for the treatment of enterobiasis. Expectations that it might be effective in the treatment of protozoal infections were not fulfilled. Instead, Henri Laborit, a surgeon in the French Navy, at the Bizerte Naval Hospital in Sidi-Abdallah, Tunisia, found promethazine, one of the antihistaminic phenothiazines synthesized in the early 1940s, to be eminently suited for the prevention of surgical shock.[56,57] It produced "euphoric

quietude" with a "state of indifference" and when given prior to surgery patients remained "calm, somewhat somnolent, and relaxed."[58]

In 1950 Laborit moved from Bizerte to Paris and asked Dr Beal from the administration of Rhône-Poulenc for a somewhat similar phenothiazine to promethazine that could hopefully attenuate patients' anxiety while potentiating anesthesia. In 1951 he received a supply of CPZ for his clinical investigations. In February 1952 Laborit, in collaboration with Huguenard and Alluaume, reported that in doses of 50 to 100 mg intravenously, CPZ does not cause loss of consciousness or any change in the patient's mentation, but produces a tendency to sleep and disinterest in the surroundings.[57] In the same report Laborit recognized the potential use of CPZ in psychiatry.[59]

The first use of CPZ in a psychiatric patient was reported by Hamon, Paraire, and Velluz, at Val de Grace, the military hospital in Paris, in March 1952, about a month after the report of Laborit.[60] Before the end of the year there were several other reports, including the six papers by Delay and Deniker from the Saint Anne Hospital in Paris that set the stage for CPZ's development in psychiatry; there followed a report on the successful treatment of an aggressive paranoid patient by Follin, at Montauban Mental Hospital, in France, and an article on 20 psychiatric patients treated with CPZ, by Rigotti, in Padua, Italy. CPZ became available on prescription in France in November 1952 under the trade name of Largactil. Subsequently, within a short period of 3 years, from 1953 to 1955, CPZ treatment in psychiatry spread around the world.[54,61]

The first international colloquium on the therapeutic uses of CPZ in psychiatry was held in Paris, in October, 1955, with 257 participants from 15 countries.[62] The importance of CPZ was recognized by the scientific community in 1957 with the presentation of the American Public Health Association's prestigious Albert Lasker Award to the three key players in the clinical development of the drug: Henri Laborit, for first using CPZ as a therapeutic agent and recognizing its potential for psychiatry; Pierre Deniker, for his leading role in introducing CPZ into psychiatry and demonstrating its influence on the clinical course of psychosis; and Heinz E. Lehmann, from Canada, for bringing the full practical significance of CPZ to the attention of the medical community. In the same year Daniel Bovet was awarded the Nobel Prize in Medicine for his major contributions to the synthesis of antihistamines which, through Laborit's serendipitious discovery that an antihistaminic phenothiazine, promethazine, produced a state of detachment and indifference, led to the development of CPZ.[63]

Imipramine

The serendipitous discovery of the therapeutic effect of imipramine in depression was the result of search for a CPZ-like substance for the treatment of schizophrenia by Geigy, at the time a major Swiss pharmaceutical company. The discovery is linked to the name of Roland Kuhn, a Swiss psychiatrist, working at the cantonal mental hospital of Münsterlingen.

In the mid-1950s Kuhn suggested (to Robert Domenjoz, Geigy's director of pharmacological research) the testing of G 22,355, the dibenzazepine of the company with the closest structural resemblance to CPZ, with the hope that it would have similar therapeutic effects. The basic constituent, G 22,355, is the iminodibenzyl nucleus, synthesized in 1899 by Thiele and Holzinger. Kuhn's expectations were not fulfilled. The substance was ineffective in schizophrenia. Nonetheless, before returning his drug supply, Kuhn decided to try the substance in one of his female patients with severe endogenous depression. This led to the recognition on January 18, 1956, that G 22,355 may have antidepressant effects. Encouraged by his findings, Kuhn administered G 22,355 to two more female patients with severe endogenous depression. In both patients the drug had favorable effects. Furthermore, in all three patients discontinuation of treatment resulted in relapse, which was reversed by resumption of the medication. This prompted Kuhn to treat 40 more depressed patients with G 22,355 at the clinic. It was on the basis of his observations of these patients that he concluded that the drug is effective in endogenous depression, in which vital disturbance is in the foreground.[64] Kuhn attributed his discovery to his ability to recognize the depressive population responsive to the drug. As far as he was concerned, "chance" and "good fortune" were only contributing factors.[65]

Kuhn's first paper on the treatment of depressive states with an iminobenzylderivative, G 22,355 was published in the August 31st issue of the *Swiss Medical Journal* in 1957.[66] On September 2nd, he also presented his findings at the 2nd World Congress of Psychiatry in Zurich. By the end of the year, G 22,355, the first tricyclic antidepressant, was released for clinical use in Switzerland with the generic name of imipramine, and the brand name of Tofranil.

There was strong opposition by academic psychiatry to the drug treatment of depression in the late 1950s, but Kuhn prevailed, and the introduction of imipramine opened up the path for the development of other antidepressants.

Iproniazid

In the same year that Kuhn presented and published his findings on the antidepressant effect of imipramine, two independent groups of investigators, Loomers, Saunders, and Kline, and Crane, presented their findings on the therapeutic effect of iproniazid, a monoamine oxidase inhibitor, in depression, at a regional meeting of the American Psychiatric Association in Syracuse, New York.[67,68] Iproniazid, an isonicotinic acid hydrazide, was synthesized in 1951 by Herbert Fox at Roche laboratories in Nutley, New Jersey (USA) for the chemotherapy of tuberculosis. In 1952, using iproniazid in tubercular patients, Selikoff, Robitzek, and Orenstein noted that the drug produced euphoria and overactive behavior in some patients.[69] In the same year, Zeller and his associates revealed the potent monoamine oxidase-inhibiting properties of the drug.[70]

Monoamine oxidase (MAO) is the enzyme responsible for the oxidative deamination of neurotransmitter monoamines, such as serotonin (5-HT) and norepinephrine (NE). The presence of these substances in the brain was first shown in 1953 and 1954 respectively; and the instrument (spectrophotofluorimeter),with a resolution power to measure the concentration of these monoamines and their metabolites in the brain, was introduced in 1955.[71] One year later, in 1956, Brodie, Pletscher, and Shore found an increase in brain monoamine, ie, 5-HT and NE levels, after the administration of iproniazid.[72] Nathan Kline was first to attribute the antidepressant effect of iproniazid to MAO inhibition, ie, to the rise of 5-HT and NE levels in the brain.[73]

The combination of serendipity and science that led to the development of MAO inhibitors for the treatment of depression triggered the development of neuropsychopharmacology, the scientific discipline dedicated to the study and treatment of the pathophysiology of mental syndromes with the employment of centrally acting drugs.

Sildenafil

In the current psychopharmacological era in psychiatry, the scope of psychiatry is extended to dimensional anomalies of abnormal psychology. Ever-newer drugs for multiplying indications are introduced, and in the development of at least one of these new drugs, sildenafil, serendipity has played a role.

Sildenafil is a selective 5-phosphodiesterase inhibitor that dilates cardiac vessels by acting on cyclic-GMP. However, expectations in clinical investigations with sildenafil in the treatment of angina pectoris conducted by Pfizer, one of the major American pharmacological companies, were not fulfilled. Instead of relieving anginal pain, the drug induced unwanted penile erections in some patients.

Independently of Pfizer, Solomon Snyder and his associates at Johns Hopkins University were working with nitric oxide (NO), a substance responsible for the physiological relaxation of blood vessels. Suspecting that NO might be a neurotransmitter, the Johns Hopkins group conducted immunochemical investigation with NO synthase (NOS), the enzyme responsible for the production of NO. In the course of this research they found that NOS is localized in the penis; demonstrated that erections are blocked by NOS inhibitors, and suggested that NO is the transmitter of penile erection.[74] Since the action of NO is mediated by cyclic GMP, similar to that of sildenafil, the side effect of penile erection, reported by cardiac patients in the Pfizer study, was explained[75] by the findings of the Hopkins group.

Shifting the direction of clinical investigations with sildenafil from angina pectoris to erectile dysfunction led to the demonstration of the effectiveness of the drug in the treatment of male erectile disorder (*Diagnostic and Statistical Manual of Mental Disorders*, 4th ed - *DSM-IV*[37]), and to the marketing of sildenafil with the brand name of Viagra.

Conclusions

Serendipity is one of the many contributing factors to drug discovery. It has certainly played a role in the discovery of most of the prototype psychotropic drugs.

The discovery process includes the recognition of the potential of the findings on the basis of one's knowledge and past experience. As Johann Wolfgang Goethe (1749-1832), a discoverer himself, wrote: "Discovery needs luck, invention, intellect—none can do without the other."[65] ❑

I wish to thank Dr Edward Shorter for his editorial suggestions.

El papel de la casualidad en el descubrimiento de fármacos

La casualidad es uno de los múltiples factores que pueden contribuir al descubrimiento de fármacos. Ella ha jugado un papel en el descubrimiento de fármacos psicotrópicos prototipo, los que han conducido al moderno tratamiento farmacológico en psiquiatría, y también ha jugado un papel en el descubrimiento de algunos fármacos que han tenido un impacto en el desarrollo de la psiquiatría. La "casualidad" en el descubrimiento de un fármaco implica que se encuentra algo mientras se está buscando otra cosa. Esta fue la situación en seis de los doce descubrimientos casuales revisados en este artículo: la anilina púrpura, la penicilina, la dietilamina del ácido lisérgico, el meprobamato, la clorpromazina y la imipramina. En el caso de tres fármacos como el bromuro de potasio, el hidrato de cloral y el litio, el descubrimiento fue casual debido a un razonamiento completamente falso que llevó a resultados empíricos correctos; y en el caso de otros dos fármacos como la iproniazida y el sildenafil se debió a que se encontraron valiosas indicaciones para ellos, las cuales no se habían buscado inicialmente. El descubrimiento de uno de los doce fármacos, el clordiazepóxido, fue pura suerte.

Rôle du hasard dans la découverte médicamenteuse

La "sérendipité" ou hasard est l'un des nombreux facteurs qui peuvent contribuer à la découverte médicamenteuse. Elle a joué un rôle dans la découverte de prototypes de médicaments psychotropes qui ont conduit aux traitements pharmacologiques modernes en psychiatrie. Elle a également participé à la découverte de plusieurs médicaments qui ont eu un impact sur le développement de la psychiatrie. La "sérendipité signifie d'avoir trouvé une chose alors que l'on en recherchait une autre. Cela peut s'appliquer à la découverte de médicaments. C'est ce qui s'est passé pour six des douze découvertes fortuites décrites dans cet article, comme le pourpre d'aniline, la pénicilline, le diéthylamide de l'acide lysergique (LSD), le méprobamate, la chlorpromazine et l'imipramine. Pour trois médicaments comme le bromure de potassium, l'hydrate de chloral et le lithium, la découverte fut fortuitement heureuse car un argumentaire totalement faux a abouti à des résultats empiriquement justes ; et pour deux autres, l'iproniazide et le sildénafil, les indications de ces médicaments ne sont pas celles auxquelles on avait pensé au départ. La découverte de l'un de ces douze médicaments, le chlordiazépoxide, relève du hasard pur.

REFERENCES

1. Remer T. *Serendipity and the Three Princes*. Norman, Okla: University of Oklahoma; 1965.
2. Hoffman R. Serendipity, a graceful word. Available at: http/heart-to-heart.hobby.ru/serendipity_graceful_wor.html. Accessed June 2006.
3. *Webster's Ninth Collegiate Dictionary*. Springfield Mass: Merriam-Webster Inc; 1985: 1074.
4. *Stedman's Medical Dictionary*. 25th ed. Baltimore, Hong Kong, London, Sydney: Lippincott Williams & Wilkins; 1990:1407.
5. Blackwell B. The process of discovery. In: Ayd FJ, Blackwell B, eds. *Discoveries in Biological Psychiatry*. Philadelphia, Pa; Toronto, Canada: J.B. Lippincott Company; 1970:14-15.
6. Doctor Out of Zebulon. Serendipity. *Arch Int Med*. 1963;111:385-386.
7. Golin M. Serendipity – big word in medical progress. Does "pure luck" deserve all the credit? *JAMA*. 1957;165:2084-2087.
8. Vallery-Rador R. *The Life of Pasteur*. (Devonshire RL, transl). New York, NY: Doubleday; 1924.
9. Medicine's happy accidents [editorial]. *JAMA.* 1957;165:2088-2089.
10. *Encyclopedia Britannica*. Vol 17. Perkins, Sir William Henry. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969:630.
11. Menzie E. Geschichte der Chemische Industrie in Basel. *Zeitschrift für die Chemische Industrie*. 1983;5:15-30.
12. Healy D. *The Antidepressant Era*. Cambridge, Massachusetts; London, UK: Oxford University Press; 1997:15-21.
13. *Encyclopedia Britannica*. Vol 5. Chemistry. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969:308-441.
14. Russell CA. *The History of Valency*. Oxford, UK; 1971.
15. Ban TA. Neuropsychopharmacology and the history of pharmacotherapy in psychiatry. A review of developments in the 20th century. In: Ban TA, Healy D, Shorter E, eds. *Reflections on Twentieth-Century Psychopharmacology*. Budapest, Hungary: Animula; 2004:697-720.
16. *Encyclopedia Britannica*. Vol 11. Hofmann, August Wilhelm von. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969:575.
17. *Encyclopedia Britannica*. Vol 1. Anilin. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969:950.
18. *Encyclopedia Britannica*. Vol 4. Bromine. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969:266-268.
19. Garrison FH. *An Introduction to the History of Medicine*. 4th ed. Philadelphia, Pa; London; UK: W. B. Saunders Company; 1960:466.
20. Shorter E. *A History of Psychiatry*. New York, NY; Chichester, UK Brisbane, Australia; Toronto, Canada; Singapore; Weinheim, Germany: John Wiley & Sons, Inc; 1997:190-238.
21. Lehmann HE, Ban TA. *Pharmacotherapy of Tension and Anxiety*. Springfield, Ill: Charles C. Thomas Publisher; 1970:12-13.
22. Balme RH. Early medicinal use of bromides. *J Roy Coll Physicians*. 1976;10:205-208.

23. Ewing JA, Grant WJ. The bromide hazard. *Southern Med J.* 1965;58:148-152.
24. Liebig J. Ueber die Verbindungen welche durch die Einwirkung des Chlors auf Alcohol, Aether, Olbildendes Gas und Essiggeist Entstehen. *Liebigs Annalen der Pharmazie*. 1832;1:182-230.
25. Liebreich MEP. *Das Chloral hydrate, ein neues Hypnoticum und Anaestheticum, und dessen Anwendung in der Medizin. Eine Arzneimeittel–Untersuchung*. Berlin, Germany: Müller; 1869.
26. Kline NS. Lithium: The history of its use in psychiatry. In: Kline NS, ed. *Modern Problems of Pharmacopyschiatry*. Vol 3. Basel, Switzerland; New York, NY: S. Karger; 1969;75-92.
27. Healy D. *The Creation of Psychopharmacology*. Cambridge, Mass; London, UK: Harvard University Press; 2002;47-50.
28. Garrod AB. *Gout and Rheumatic Gout.* London, UK: Walton and Maberly; 1859;438.
29. Johnson FN. *The History of Lithium*. Basingstoke, UK: MacMillan Press;1984.
30. Yeragani VK, Gershon S, Hammond WA. Lithium: a historical update. *Biol Psychiatry*. 1986;21:1101-1102.
31. Hammond WA. *A Treatise on Diseases of the Nervous System*. New York, NY: Appleton; 1871.
32. Lange C. *Om periodiske Depressionstilstande og deres Patagonese*. Copenhagen, Denmark: Jacob Lunds Forlag; 1886.
33. Cade JFJ. Lithium salts in the treatment of psychotic excitement. *Med J Aust*. 1949;2:349-352.
34. Cade JFJ. The story of lithium. In: Ayd FJ, Blackwell B, ed. *Discoveries in Biological Psychiatry*. Philadelphia, Pa; Toronto, Canada: J.B. Lippincott Company; 1970;218-229.
35. Schou M, Jüel-Nielsen N, Strömgren E, Voldby H. The treatment of manic psychosis by the administration of lithium salts. *J Neurol Neurosurg Psychiatry*. 1954;17:250-260.
36. Baastrup PC, Schou M. Lithium as a prophylactic agent: Its effect against recurrent depression and manic-depressive psychosis. *Arch Gen Psychiatry*. 1967;16:162-172.
37. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. Washington, DC: American Psychiatric Association; 1994:350-538.
38. Gjessing R. Disturbances of somatic functions in catatonia with periodic course, and their compensation. *J Ment Sci*. 1938;84:608-621.
39. Mayer-Gross W, Slater E, Roth M. *Clinical Psychiatry*. 2nd ed. London, UK: Cassell and Company Ltd; 1960:382-385.
40. Stoll A, Hofmann A. Partialsynthese von Alkaloiden Typus des Ergobasins. *Helv Chim Acta*. 1943;26:944-947.
41. Brown FC. *Hallucinogenic Drugs*. Springfield, Ill: Charles C. Thomas; 1972;36-77.
42. Jacobs WA, Craig LC. The ergot alkaloids III. On lysergic acid. *J Biol Chem*. 1934;106:393-396.
43. Hofmann A. The discovery of LSD and subsequent investigations on naturally occurring hallucinogens. In: Ayd FJ, Blackwell B, ed. *Discoveries in Biological Psychiatry*. Philadelphia, Pa;Toronto, Canada: J.B. Lippincott Company; 1970;91-106.
44. *Encyclopedia Britannica* Vol 9. Fleming, Sir Alexander. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969;437.
45. Beveridge WIB. *The Art of Scientific Investigation*. New York, NY: W.W. Norton Company; 1957;162.
46. *Encyclopedia Britannica* Vol 17. Penicillin. Chicago, Ill; London, UK; Toronto, Canada; Geneva, Switzerland; Sydney, Australia; Tokyo, Japan; Manila, the Philippines: William Benton; 1969;533-534.
47. Stokes JH, Sternberg TH, Schwartz WH, Mahoney JF, Moore JE, Wood WB. The action of penicillin in late syphilis including neurosyphilis. *JAMA*. 1944;126:73-79.
48. Berger FM. As I remember. In: Ban TA, Healy D, Shorter E, eds. *The Rise of Psychopharmacology and the Story of CINP*. Budapest, Hungary: Animula; 1998;59-62.
49. Berger FM, Bradley W. The pharmacological properties of a ,-dihydroxy-Á-(2-methylphenoxy)-propane (Myanesin). *Br J Pharmacol*. 1946;1:265-272.
50. Ban TA. *Psychopharmacology*. Baltimore, Md: Williams and Wilkins; 1969;313-325.
51. Berger FM. Anxiety and the discovery of the tranquilizers. In: Ayd FJ, Blackwell B, eds. *Discoveries in Biological Psychiatry*. Philadelphia, Pa; Toronto, Canada: J.B. Lippincott Company; 1970;115-129.
52. Burger A. History. In: Usdin E, Forrest IS. *Psychotherapeutic Drugs. Part I Principles.* New York, NY; Basel, Switzerland: Marcel Dekker, Inc; 1976;11-57.
53. Greenblatt DJ, Shader RI. *Benzodiazepines in Clinical Practice*. New York, NY: Raven Press; 1974:263-264.
54. Cohen IM. The benzodiazepines. In: Ayd FJ, Blackwell B, eds. *Discoveries in Biological Psychiatry*. Philadelphia, Pa; Toronto, Canada: J.B. Lippincott Company; 1970;110-141.
55. Caldwell AE. *Origins of Psychopharmacology From CPZ to LSD*. Springfield, Ill: Charles C. Thomas; 1970:3-134.
56. Caldwell AE. History of psychopharmacology. In: Clark WG, del Giudice J, eds. *Principles of Psychopharmacology*. New York, NY; London, UK: Academic Press; 9-30.
57. Laborit H. Henri Laborit. In: Ban TA, Ray OS, eds. *A History of the CINP*. Brentwood, Tn: J.M. Productions; 1996;218-221.
58. Laborit H. Étude expérimentale du syndrome d'irritation et application clinique à la maladie post-traumatique. *Thérapie*. 1949;4:126-139.
59. Laborit H, Huguenard P, Alluaume R. Un nouveau stabilisateur végétatif (le 4560 RP). *Presse Méd*. 1952;60:37-348.
60. Hamon J, Paraire J, Velluz J. Remarques sur l'action du 4560 RP sur l'agitation maniaque. *Annales Medicopsychologiques* (Paris). 1952;110: 331-335.
61. Ban TA. *Schizophrenia. A Psychopharmacological Approach*. Springfield, Ill: Charles C. Thomas; 1971;3-4.
62. Hollister LE. Review of the International Colloquium in Chlorpromazine. In: Ban TA, Ray OS, eds. *A History of the CINP*. Brentwood, Tn: J.M. Productions; 1996;275-280.
63. Ban TA. Nobel Prize and Albert Lasker Award. In: Ban TA, Ray OS, eds. *A History of the CINP*. Brentwood, Tn: J.M. Productions; 1996:265-271.
64. Kuhn R. The discovery of the tricyclic antidepressants and the history of their use in early years. In: Ban TA, Ray OS, eds. *A History of the CINP*. Brentwood, Tn: J.M. Productions; 1996:425-435.
65. Kuhn R. The imipramine story. In: Ayd FJ, Blackwell B. *Discoveries in Biological Psychiatry*. Philadelphia, Pa; Toronto, Canada: J.B. Lippincott Company; 205-217.
66. Kuhn R. Über die Behandlung depressiver Zustände mit einem iminodibenzylderivat (G 22, 355). *Schweiz Med Wsch*. 1957;87:1135-1140.
67. Loomer HP, Sanders JC, Kline NS. A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. *Psychiatric Res Reports*. 1957;8:129-141.
68. Crane GE. Iproniazid (Marsilid) phosphate: a therapeutic agent for mental disorders. *Psychiatric Res Rep*. 1957;8142-154.
69. Selikoff IJ, Robitzek EH, Orenstein GG. Treatment of pulmonary tuberculosis with hydrazine derivatives of isonicotinic acid. *JAMA*. 1952;150:973-980.
70. Zeller EA, Barsky J, Fouts JR, Kirschheimer WF, Van Orden LS. Influence of isonicotinic acid hydrazide (INH) and isonicotinyl-2-isopropyl hydrazide (IIH) on bacterial and mammalian enzymes. *Experientia*. 1952;8:349-350.
71. Bowman RL, Caulfield PA, Udenfriend S. Spectrophotofluorimetry in the visible and ultraviolet. *Science*. 1955;122:32-33.
72. Brodie BB, Pletscher A, Shore PA. Possible role of serotonin in brain function and in reserpine action. *J Pharmacol Exp Ther*. 1956;9:126-127.
73. Berger PA, Barchas JD. Monoamine oxidase inhibitors. In: Usdin E, Forrest IS, eds. *Psychotherapeutic Drugs. Part II Applications*. New York, NY: Basel, Switzerland: Marcel Dekker, Inc; 1977;1173-1216.
74. Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide; a physiologic mediator of penile erection. *Science.1992;257:401-403*.
75. Snyder SH. Forty years of neurotransmitters. In: Ban TA, Healy D, Shorter E, eds. *From Psychopharmacology to Neuropsychopharmacology in the 1980s and the Story of CINP As Told in Autobiography*. Budapest, Hungary: Animula; 2002;36-42.

Surrogate outcomes in neurology, psychiatry, and psychopharmacology

Luc Staner, MD

A surrogate outcome can be defined as an outcome that can be observed sooner, at lower cost, or less invasively than the true outcome, and that enables valid inferences about the effect of intervention on the true outcome. There is increasing interest in the use of surrogate outcomes of treatment efficacy measurement in investigational drug trials. However, the significance of surrogate markers of treatment outcome in neurology and psychiatry has not yet been sufficiently demonstrated. Few such markers have been adequately "validated," that is, shown to predict the effect of the treatment on the clinical outcome of interest. In this article, evidence that would support the validation of such markers is discussed. Biomarkers used during early clinical development programs of new psychotropic compounds are considered in the contexts of Parkinson's disease, affective disorder, and schizophrenia. The particular case of neuroprotective trials is exemplified by Parkinson's disease, where a biomarker substituting for a clinical measure of progression could be considered as a surrogate treatment outcome.

 Dialogues Clin Neurosci. 2006;8:345-352.

Keywords: *Surrogate outcome; biomarker; Parkinson's disease; affective disorder; schizophrenia*

Author affiliations: Centre Hospitalier, Secteur VIII, Rouffach, France

Clinicians making treatment decisions generally refer to methodologically strong clinical trials examining the impact of therapy on patient-important outcomes such as morbid end points, ie, stroke, myocardial infarction, and death, or health-related quality of life end points. These trials require such a large sample size or long patient follow-up that researchers have proposed the alternative of substituting surrogate outcomes or end points for the target event, allowing shorter and smaller trials to be conducted. This offers an apparently simpler solution to the difficulty of conducting large or long-term trials.

A surrogate outcome can be defined as an outcome that can be observed sooner, at lower cost, or less invasively than the true outcome, and that enables valid inferences about the effect of intervention on the true outcome.

Surrogate outcomes or end points (also known as surrogate markers) have to be distinguished from biomarkers, although the two concepts are related. According to the Biomarker Definitions Working Group,[1] a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic process, or pharmacologic responses to a therapeutic intervention." According to the US Food and Drug Administration (FDA)[2] a "valid biomarker" is one "that is measured in an analytical test system with well-established performance characteristics and for which there is an established framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic or clinical significance of the test result." Thus, in the drug development process, biomarkers can be useful tools from the discovery stage, where they are used to investigate pathophysiologic mechanisms related to

Address for correspondence: Dr L. Staner, Centre Hospitalier, 27 rue du 4RSM, F-68250 Rouffach, France
(e-mail: luc.staner@ch-rouffach.fr)

Selected abbreviations and acronyms

FDA	*Food and Drug Administration*
fMRI	*functional magnetic resonance imaging*
PET	*positron emission tomography*
PSA	*prostate-specific antigen*
REM	*rapid eye movement*
SPECT	*single photon-emission computed tomography*

either diagnosis or prognosis of a disease, through the later stages of clinical development. Biomarkers can be used in preclinical studies to confirm in vivo activity as well as to investigate dose-response relationships. During early clinical development programs (phase 1 and 2a), biomarkers are used to evaluate activity and to develop pharmacokinetic-pharmacodynamic relationships. In phase 3 and 4 studies, biomarkers are useful tools for stratifying study populations.

Surrogate outcomes are biomarkers that fit the following definition: "a biomarker that is intended to substitute for a clinical end point. A surrogate end point is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence."[1] This definition of a surrogate outcome illustrates the key difference versus the role of the biomarker. A biomarker can be used as a surrogate outcome if it can reasonably predict a clinical benefit. Thus, although a surrogate outcome is by definition a biomarker, in fact, a very small minority of biomarkers meet the standard of a surrogate outcome. Before 1991, regulatory agencies such as the FDA used surrogate treatment outcomes in limited settings, mainly in the cardiovascular area. For example, antihypertensive drugs have been approved for marketing based on their effectiveness in lowering blood pressure, and cholesterol-lowering agents have been approved based on their ability to decrease serum cholesterol, not on the direct evidence that they decrease mortality from cardiovascular diseases. In 1991, during the acquired immune deficiency syndrome (AIDS) epidemic, surrogate outcomes were utilized for the first time as a viable path toward regulatory approval. Indeed, an important milestone was the use of CD4 cell count as a surrogate marker, because of its predictive value for outcome. This led to the approval of didanosine for the treatment of HIV. In 1992, the FDA formulated a new regulatory process, often referred to as "accelerated approval," under which marketing approval can be provided for interventions that have been shown to have compelling effects on a validated surrogate treatment outcome.

At the present time, there are well-defined procedures in the FDA in which such approvals are routinely examined[3]; for example, some anticancer treatments have been approved under the accelerated approval regulations.[3] In these cases, drugs tested in patients refractory to available treatments are approved on the basis of their effects on tumor size, as assessed by imaging. The labeling adopted at the time of the approval indicates that the approval was based on the effects of the treatment on tumor size, without evidence of an effect on other clinical variables. It is only when subsequent studies demonstrate an effect on clinical outcome that the labeling is changed to include a description of the documented effect on survival.

In the field of drugs acting on the central nervous system (CNS), no treatments for neurologic or psychiatric diseases have been approved to date on the basis of an effect on a surrogate outcome. One obvious reason for this is the fact that no surrogate outcomes have been validated until now; this will be discussed in the next section.

Surrogate outcome validation

The presence of a correlation does not suffice to justify the replacement of a true clinical outcome by a surrogate marker of this outcome. Indeed, a surrogate outcome might not involve the same pathophysiologic process that results in the true clinical outcome. In oncology, an elevated level of a tumor marker such as prostate-specific antigen (PSA) in prostate cancer is the indication of an advanced tumor stage, and is clearly correlated with morbidity/mortality risks. However, PSA is not the mechanism through which the disease process influences the clinical outcome. It is thus questionable whether treatment-induced changes in this marker accurately predict treatment-induced effects on the clinical end points.[4,5]

General guidelines for the interpretation of clinical trials using surrogate outcomes have been proposed.[6] In a recent paper, Fleming[7] suggested a four-level hierarchy for outcome measures.

Level 1 is a true clinical efficacy measure, and includes those outcomes that directly reflect real benefits for the patient; for example, reducing the risk of stroke could be a surrogate for reducing the risk of death.

Level 2 is a validated surrogate outcome for a specific disease setting and class of intervention. This outcome, while not directly representing tangible clinical benefits, can be used to reliably predict the level of such benefits.

An example is blood pressure reduction as a surrogate risk for stroke, for a well-studied class of antihypertensive agents.

Level 3 is a nonvalidated surrogate outcome, yet one established to be reasonably likely to predict clinical benefit for a specific disease setting and class of intervention. "Reasonably likely" implicates considerable clinical evidence that the effect of the intervention on the surrogate outcome measure (i) will accurately represent the effect of the intervention on what is thought to be the predominant mechanism through which the disease process induces tangible events; (ii) does not have important adverse effects on the clinical efficacy end point that would not be detected by the outcome measure; (iii) is consistent with the effects on the true clinical outcome; and (iv) is sufficiently strong and durable that it is reasonably likely to product meaningful clinical benefits on clinical efficacy measures. Illustrations of this level 3 of outcome measures would be a reduction in viral load to an undetectable level for 6 months in patients with advanced HIV infection.

Level 4 is a correlate outcome that is a measure of biological activity, but that has not been established to be at a higher level in this four-level hierarchy for outcome measures; biological markers, such as PSA, that almost certainly do not represent the biological mechanism through which the disease process induces clinically tangible events, would tend to be at level 4.

Marketing approval under the accelerated approval process can be provided for interventions having compelling effects on biological markers that are at least at level 3 in the hierarchy. In the field of drugs acting on the CNS, to date no compounds have been approved with the accelerated approval procedure on the basis of an effect on a surrogate outcome. This highlights the lack of strongly validated (ie, level 1, 2, or 3) surrogate outcomes in the field of neurology and psychiatry. The following sections will focus on definitions, applications, successes, and failures of biomarkers in Parkinson's disease, affective disorder, and schizophrenia, although similar examples could be found for many other neurological or psychiatric disorders.

Neurology: Parkinson's disease

Parkinson's disease is a progressive neurodegenerative disorder characterized by rigidity, bradykinesia, postural instability, and tremor. Clinical decline reflects the ongoing degeneration of dopaminergic neurons. Development of specific biomarkers for Parkinson's disease may be useful at the onset of neurodegeneration, the onset of disease, and/or to mark disease progression. At present, the most mature surrogate measures for Parkinson's disease are based on the functional imaging of dopaminergic neurons with dopamine transporter ligands on the measures of dopamine metabolism with fluorodopa.[8,9] 2-β-Carbomethoxy-3-β-(4-[^{125}I]iodophenyl)tropane (^{123}I-b-CIT), a single photon-emission computed tomography (SPECT) radioligand that binds to the dopamine transporter on the presynaptic dopamine terminal,[10] has been most extensively evaluated as a potential surrogate outcome in Parkinson's disease. It has been reliably shown to distinguish healthy control subjects from parkinsonian patients.[11] Moreover, longitudinal studies reveal an annual 6% to 10% reduction in striatal dopamine transporter as measured by ^{123}I-b-CIT uptake in early Parkinson's disease, with a slower decline in more advanced disease.[9,12] However, the results of CALM-PD trial and the ELLDOPA trial contradicted these results. In the CALM-PD trial, subjects with early Parkinson's disease requiring dopaminergic therapy were randomized to either initial pramipexole or initial levodopa.[13] A subgroup of patients (n=28) were studied in terms of rate of striatal dopamine transporter loss as measured by SPECT ^{123}I-b-CIT uptake.[14] Results show that, over the course of 46 months, the pramipexole-treated patients showed a 16% decline in striatal uptake compared with 25% in the levodopa group. The biomarker advantage of pramipexole, however, did not translate into a clear, clinically meaningful advantage. Indeed, although patients on pramipexole had a lower incidence of complications, patients randomized to initial levodopa had an early and sustained improvement in function, and less somnolence and edema. In the ELLDOPA trial[15] during which three increasing doses of levodopa were compared with placebo in patients with early Parkinson's disease not requiring dopaminergic therapy, discordant results were noted between the clinical outcomes and the neuroimaging end point. Analysis of the ^{123}I-b-CIT outcome suggested a trend toward a more rapid decline in striatal dopamine transporter in individuals on the highest doses of levodopa, but the largest clinical improvement was observed in the levodopa group, in the direction opposite to what would be predicted on the basis of the imaging marker. These results corroborate those of the CALM-PD trial, and indicate that the SPECT ^{123}I-b-CIT

biomarker advantage did not translate into a clinically meaningful advantage.

Studies using ^{18}F6-fluoro-L-dopa (F-dopa) positron emission tomography (PET) as a surrogate outcome of Parkinson's disease treatment show similar negative results. The accumulation of these radioactive dopamine metabolites within the striatum, and evidence correlating their reduction with clinical and pathologic measures,[16-18] make F-dopa PET a potential surrogate outcome for treatment assessment. In the REAL PET trial, 2 years after starting treatment, a 13% decline in F-dopa uptake was seen in the ropinirole group compared with a 20% decline in the levodopa group.[19] However, patients treated with levodopa had significantly greater functional improvement and fewer side effects (excepting dyskinesia), suggesting that F-dopa PET did not meet criteria for a surrogate outcome of treatment efficacy. Additional concerns regarding the ability to utilize PET as a marker of therapeutic efficacy come from studies evaluating the safety and efficacy of fetal tissue transplantation.[20-22] In these studies, a significant increase in F-dopa uptake was demonstrated in patients receiving fetal tissue transplantation. Regrettably, functional improvement was not clearly established, and a significant proportion of treated subjects in both studies developed disabling dyskinesias. This is a clear example of a case where unexpected consequences of an intervention, not detected by a potential surrogate outcome, resulted in patient harm.

The negative results of these trials have raised questions regarding the use of biomarkers in Parkinson's disease. How can drugs affect a biomarker that suggests a slowing of disease progression in the absence of symptomatic benefits? How should symptomatic benefits in the absence of disease-modifying effects be weighed against modest neuroprotective effects in the absence of symptomatic benefits? Are biomarkers any better than clinical measures?

Some of these questions are being addressed in neuroprotective trials for Parkinson's disease. The rationale for these trials relies on the fact that in vitro and in vivo studies have established that there is abnormal oxidative stress in Parkinson's disease.[23-25] The link between this particular disease mechanism and the clinical symptoms of the illness, however, is weak, and the goal of the trial is to detect no change in clinical status; even a worsening in clinical status could be considered a success if the rate of worsening is slower than expected. On the other hand, an improvement in clinical status is considered as a potential confounding factor since it may not relate to the neuroprotective potency of the drug but, for instance, to direct effects on the synaptic transmission. This is illustrated by the DATATOP study,[26] a trial designed with the hypothesis that deprenyl, a monoamine oxidase B inhibitor, the antioxidant α-tocopherol, and the combination of the two compounds, might slow the clinical progression of the disease. The results showed that patients on deprenyl were found to be less likely to require dopaminergic therapy over time, a finding that could be interpreted as evidence of a neuroprotective effect in cases of unaltered clinical status. However, the reason for the difference was that deprenyl produced a small but statistically significant symptomatic benefit, casting doubts about its neuroprotective effect.[27] Accordingly, the DATATOP study demonstrates that, in trials assessing the effects of a neuroprotective drug, clinical measures cannot be considered as a gold standard for measuring disease progression. In this particular case, a biomarker directly reflecting disease progression could be substituted for a clinical measure of progression.

Psychiatry: affective disorder and schizophrenia

Clinical outcome measures in psychiatry provide several challenges for drug developers. Periods of several weeks or longer can be necessary to detect a response. Often, assessments are obtained from rating scales, which are based on psychometric, rather than pathophysiological, principles. Moreover, placebo response rates are high for many indications. Surrogate measures be applied to overcome these difficulties, but research in this field is still in its infancy. One may acknowledge that, compared with some neurological diseases such as Parkinson's disease, illness-specific biomarkers are more poorly defined in psychiatry. In this context, defining surrogate treatment outcomes in psychiatry is premature to say the least. At the present time, only a few biomarkers have been proposed as surrogate outcomes for screening of new drugs in early clinical phases. Accordingly, this discussion is focused on biomarkers of potential interest.

Affective disorder

Affective disorder is characterized by episodes of depression and in some cases, of mania, that recur and remit repeatedly and cause shifts in a person's mood, energy,

and ability to function. In the most severe form of affective disorder, ie, bipolar disorder, patients experience cycling of moods that usually swing from being overly elated or irritable to sad and hopeless and then back again, with periods of normal mood in between. Unequivocally validated biomarkers for affective disorder are sparse; there are, however, studies suggesting that measurement of stress hormone regulation processes, of rapid eye movement (REM) sleep or of functional magnetic resonance imaging (fMRI) activation of limbic areas could represent valuable surrogate outcome of pharmacological antidepressant activity. Stress-related dysfunctional neuroendocrine regulation implicating the corticotropin-releasing hormone (CRH) system has been consistently demonstrated in major depression,[28,29] and it has been proposed that neurodocrine dynamic challenge tests such as the combined dex/CRH test serve as a screening tool to demonstrate the antidepressive effects of new compounds in clinical drug trials.[30,31]

Indices of REM sleep disinhibition, such as shortened latency to REM sleep and increased density of ocular movement during REM sleep, have been proposed as a familial sleep biomarker for increased risk of developing depression.[32] Indeed, many studies, recently reviewed,[33] suggest that REM sleep disinhibition could reflect a dysfunction of the monoaminergic system involved in the pathophysiology of affective disorder. Drugs increasing noradrenergic or serotoninergic functions inhibit REM sleep, a property shared by most antidepressant drugs. Consequently, REM sleep inhibition has been proposed as a potential biomarker of the antidepressant activity of a compound.[34,35]

Dysfunction of the prefrontal cortex, including the ventral anterior cingulate gyrus, has been implicated in anhedonia, exaggerated response to stress, abnormal response after presentation of mood-lowering stimuli, serotoninergic challenges (such as tryptophan depletion paradigms), or selective serotonin reuptake inhibitor (SSRI) administration (reviewed by Hassler et al[36]). Changes in anterior cingulate function during affective facial processing associated with symptomatic improvement indicate that such an fMRI activation paradigm may be a useful surrogate outcome of antidepressant treatment response.[37] Another area of interest whose dysfunctional activation could serve as a surrogate outcome of antidepressant activity is the amygdala. Affective disorders have been characterized by an increased basal metabolism of the amygdala[38] that seems to relate to hypercortisolism and REM sleep abnormalities.[37] Increased amygdala reactivity in response to fearful stimuli has been observed in healthy individuals with a susceptibility to affective disorders.[39-41] Moreover, a recent study in healthy volunteers showed that antidepressant administration decreases amygdala responses to the presentation of fearful stimuli.[42] This indicates that the amygdala response to fearful stimuli, even in healthy subjects, could represent a surrogate outcome of the pharmacological effects of antidepressants.

Schizophrenia

Schizophrenia is a chronic psychiatric illness manifested by characteristic and severe distortions of thinking and perception, and by inappropriate or blunted affect. Symptoms of schizophrenia may be divided into positive symptoms (including hallucinations, delusions, and disorganized speech and behavior), negative symptoms (including a decrease in emotional range, poverty of speech, loss of interests, and loss of drive) and cognitive symptoms (including deficits in attention and executive functions such as organizational ability and abstract thinking). The diagnosis is made from a pattern of signs and symptoms, in conjunction with impaired occupational or social functioning. As for affective disorder, there are, at the present time, no surrogate treatment outcomes for schizophrenia. Some biomarkers have been proposed as tools for the development of new antipsychotic drugs, and will be further discussed.

Abnormal evoked response electronecephalography (EEG) potentials have been shown to characterize patients with schizophrenia, and are suggested to reflect disturbances of neuropsychological functioning. In this model, it is believed that schizophrenia patients are overwhelmed by sensory input that they have trouble organizing, due to a deficit in the filtering or the gating process of extraneous sensory stimuli.[43,44] Among the several methods that have been used to investigate this putative deficit in inhibitory neuronal processing, we will focus on the two most widely used techniques, P50 auditory sensory gating and the prepulse inhibition of the acoustic startle response. Abnormal P50 and prepulse inhibition responses have been observed in patients with schizophrenia and in their families.[44,45] The P50 is a small-amplitude, positive event-related potential that occurs about 50 msec after an auditory stimulus. Repeated pairs of clicks, separated by about 500 msec, typically elicit an initial excitatory response followed by a diminished response, because the inhibitory

mechanism activated by the first stimulus interferes with the excitatory response to the second stimulus. The percentage reduction in the amplitude of the P50 response from the first to the second click is the dependent variable labeled "P50 suppression." Significantly lower suppression is found in schizophrenia patients.[44-47] Interestingly, treatment with clozapine, but not with conventional antipsychotic drugs such as haloperidol, reverses this deficit.[48] Moreover, subsequent studies have shown that other atypical antipsychotic medication did not share this property with clozapine[49] and that improvement in P50 sensory gating was a predictor of clozapine response in schizophrenia patients.[50] These findings suggest that P50 could be a valuable biomarker for the development of new antipsychotic agents, given the fact that clozapine is clinically more effective in a significant proportion of schizophrenic patients refractory to other drug treatment.

Another auditory electrophysiological parameter assessing sensorimotor gating is the prepulse inhibition of the acoustic startle response. It refers to the ability of a weak (prepulse) stimulus to transiently inhibit the reflex response to a closely following stronger (pulse) stimulus. Prepulse inhibition deficits have been observed in patients with schizophrenia[44,45] including in drug-naïve patients.[51,52] In rats, prepulse inhibition is disrupted by systemic administration of dopamine agonists, serotonin agonists, or glutamate antagonists, and this paradigm has been proposed as an animal model for predicting antipsychotic activity of novel compounds.[53] As for P50 suppression, there is preliminary evidence suggesting that, in contrast to other antipsychotic drugs including atypical antipsychotics, clozapine treatment improves the prepulse inhibition deficits of schizophrenic patients.[54] This indicates that indices of sensorimotor gating deficit measured by either P50 or prepulse inhibition paradigms are interesting biomarkers for the development of new clozapine-like antipsychotic drugs.

Conclusions

At this time, the significance of surrogate markers of treatment outcome in neurology and psychiatry is not yet sufficiently understood; moreover, no surrogate markers have been validated to be used as a sole primary measure of effectiveness in trials of investigational drugs. Although unvalidated (in the sense described earlier) surrogate outcomes have been successfully used for anticancer or anti-AIDS drugs, a sponsor who wishes to obtain approval on the basis of the effect of a drug on such an unvalidated marker will need to adequately demonstrate that any such effect will be "reasonably likely" to predict the desired clinical effect. Evidence supporting this remains to be found. It may include both animal and human data, and requires further investigation into the pathophysiology of the condition under study and into the pharmacology of the drug under study. ❑

REFERENCES

1. Biomarkers Definition Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharm Ther*. 2001;69:89-95.
2. Woodcock J. Presentation of FDA Document, *Draft Guidance for Industry: Pharmacogenomic Data Submission*. 13 November 2003. (See section 506[b] of the Act (21 U.S.C. sec.356]; see also Guidance for Industry: Fast Track Drug Development Programs--Designation, Development, and Application Review, Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research [September 1998] at 2 ["Section 506(b) essentially codifies in statute FDA's accelerated approval regulations."]).
3. Barry MJ. PSA screening for prostate cancer: the current controversy-a viewpoint. Patient Outcomes Research Team for Prostatic Diseases. *Ann Oncol*. 1998;9:1279-1282.
4. Bunting PS. Screening for prostate cancer with prostate-specific antigen: beware the biases. *Clin Chim Acta*. 2002;315:71-97.
5. Johnson JR, Williams G, Pazdur R. End points and United States Food and Drug Administration approval of oncology drugs. *J Clin Oncol*. 2003;21:1404-1411.
6. Bucher HC, Guyatt GH, Cook DJ, Holbrook A, McAlister FA. Users' guides to the medical literature. XIX. Applying clinical trial results. A. How to use an article measuring the effect of an intervention on surrogate end points. Evidence-Based Medicine Working Group. *JAMA*. 1999;282:771-778.
7. Fleming TR. Surrogate endpoints and FDA's accelerated approval process. *Health Affairs*. 2005;24:67-78.
8. Burn DJ, Sawle GV, Brooks DJ. Differential diagnosis of Parkinson's disease, multiple system atrophy, and Steele-Richardson-Olszewski syndrome: discriminant analysis of striatal 18F-dopa PET data. *J Neurol Neurosurg Psychiatry*. 1994;57:278-284.
9. Marek K, Innis R, van Dyck C, et al. [123I]beta-CIT SPECT imaging assessment of the rate of Parkinson's disease progression. *Neurology*. 2001;57:2089-2094.
10. Marek K, Jennings D, Seibyl J. Single-photon emission tomography and dopamine transporter imaging in Parkinson's disease. *Adv Neurol*. 2003;91:183-191.
11. Parkinson Study Group. A multicenter assessment of dopamine transporter imaging with DOPASCAN/SPECT in parkinsonism. *Neurology*. 2001;57(10 suppl 3):S52-S59.
12. Pirker W, Djamshidian S, Asenbaum S, et al. Progression of dopaminergic degeneration in Parkinson's disease and atypical Parkinsonism: a longitudinal b-CIT SPECT study. *Mov Disord*. 2002;17:45-53.

Mediciones sustitutas en neurología, psiquiatría y psicofarmacología

Una medición sustituta se puede definir como un parámetro (marcador) mensurable en forma precoz, a un menor costo y de manera menos invasora que el parámetro real; y que permite inferencias válidas acerca del efecto de la intervención en el parámetro real. Existe un creciente interés en el empleo de mediciones sustitutas de la eficacia terapéutica en investigación de ensayos de fármacos. Sin embargo, el significado de mediciones sustitutas de eficacia terapéutica en neurología y psiquiatría aun no ha sido suficientemente demostrado. De hecho, pocos de estos marcadores han sido adecuadamente "validados," es decir, que hayan mostrado su valor predictor en relación al efecto clínico de un determinado tratamiento. En este artículo se discute la evidencia que podría sustentar la validación de tales marcadores. Los biomarcadores empleados durante los programas de desarrollo iniciales de nuevos fármacos psicotrópicos son considerados en el contexto de la enfermedad de Parkinson, los trastornos afectivos y la esquizofrenia. El caso particular de los ensayos de moléculas neuroprotectoras está ejemplificado en la enfermedad de Parkinson, donde un biomarcador sustituto para la medición clínica de la progresión de la enfermedad podría considerarse como una medición sustituta de la eficacia del tratamiento.

Marqueurs de substitution en neurologie, psychiatrie et psychopharmacologie

Un marqueur de substitution peut se définir comme un paramètre (marqueur) mesurable de façon plus précoce, ou moins invasive, ou encore pour un coût moindre que le paramètre réel, et qui permet de tirer des conclusions valides sur l'effet d'une thérapeutique sur ce paramètre réel. Un intérêt croissant se porte sur l'utilisation de tels marqueurs de substitution dans le domaine des études d'évaluation clinique des médicaments. Toutefois, la pertinence des marqueurs de substitution d'efficacité n'a pas encore été démontrée de façon satisfaisante dans les domaines de la neurologie et de la psychiatrie. En effet, peu parmi ces marqueurs ont été suffisamment « validés », c'est-à-dire, ont fait la preuve de leur valeur prédictive quant à l'effet clinique recherché du traitement. Cet article passe en revue les arguments en faveur de la validation de ce type de marqueur. Les biomarqueurs utilisés dans les programmes de développement initiaux de nouvelles molécules psychotropes sont évoqués dans le contexte de la maladie de Parkinson, des troubles affectifs et de la schizophrénie. Le cas particulier des essais cliniques de molécules à visée neuroprotectrice est illustré par la maladie de Parkinson, où un biomarqueur de la progression de la maladie pourrait être considéré comme marqueur de substitution du résultat thérapeutique.

13. Parkinson Study Group. Pramipexole vs levodopa as initial treatment for Parkinson disease: a randomized controlled trial. *JAMA*. 2000;284:1931-1938.
14. Parkinson Study Group. Dopamine transporter brain imaging to assess the effects of pramipexole vs levodopa on parkinson's disease progression. *JAMA*. 2002;287:1653-1661.
15. Fahn S, Oakes D, Shoulson I, et al. Levodopa and the progression of Parkinson's disease. *N Engl J Med*. 2005;351:2498-2508.
16. Vingerhoets FJ, Snow BJ, Lee CS, Schulzer M, Mak E, Calne DB. Longitudinal fluorodopa positron emission tomographic studies of the evolution of idiopathic parkinsonism. *Ann Neurol*. 1994;36:759-764.
17. Snow BJ, Tooyama I, McGeer EG, et al: Human positron emission tomographic [18F]fluorodopa studies correlate with dopamine cell counts and levels. *Ann Neurol*. 1993;34:324-330.
18. Ribeiro MJ, Vidailhet M, Loc'h C, et al. Dopaminergic function and dopamine transporter binding assessed with positron emission tomography in Parkinson's disease. *Arch Neurol*. 2002;59:580-586.
19. Whone AL, Watts RL, Stoessl AJ, et al. Slower progression of Parkinson's disease with ropinirole versus levodopa: The REAL-PET study. *Ann Neurol*. 2003;54:93-101.
20. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med*. 2001;344:710-719.
21. Olanow CW, Kieburtz K, Stern M, et al. Double-blind, placebo-controlled study of entacapone in levodopa-treated patients with stable Parkinson disease. *Arch Neurol*. 2004;61:1563-1568.
22. Hagell P, Cenci MA. Dyskinesias and dopamine cell replacement in Parkinson's disease: a clinical perspective. *Brain Res Bull*. 2005;68:4-15.
23. Zhang Y, Dawson VL, Dawson TM. Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiol Dis*. 2000;7:240-250.
24. Youdim MB, Grunblatt E, Levites-Royak Y, Mandel S. Drugs to prevent cell death in Parkinson's disease. Neuroprotection against oxidative stress and inflammatory gene expression. *Adv Neurol*. 2001;86:115-124.
25. Schapira AH. Disease modification in Parkinson's disease. *Lancet Neurol*. 2004;3:362-368.
26. Parkinson Study Group. DATATOP: a multicenter controlled clinical trial in early Parkinson's disease. *Arch Neurol*. 1989;46:1052-1060.
27. Landau WM. Clinical neuromythology IX. Pyramid sale in the bucket shop: DATATOP bottoms out. *Neurology*. 1990;40:1337-1339.

28. Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*. 2000;23:477-501.
29. Müller MB, Wurst W. Getting closer to affective disorders: the role of the CRH receptor system. *Trends Mol Med*. 2004;10:409-415.
30. Duval F, Mokrani MC, Ortiz JA, Schulz P, Champeval C, Macher JP. Neuroendocrine predictors of the evolution of depression. *Dialogues Clin Neurosci*. 2005;7:273-282.
31. Ising M, Kunzel HE, Binder EB, Nickel T, Modell S, Holsboer F. The combined dexamethasone/CRH test as a potential surrogate marker in depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005;29:1085-1093.
32. Modell S, Ising M, Holsboer F, Lauer CJ. The Munich vulnerability study on affective disorders: premorbid polysomnographic profile of affected high-risk probands. *Biol Psychiatry*. 2005;58:694-699.
33. Staner L, Luthringer R, Le Bon O. Sleep disturbances in affective disorders. In: Pandi-Perumal SR, Monti JM, eds. *Clinical Pharmacology of Sleep,* Basel, Switzerland: Birkhaüser Verlag; 2006:101-124.
34. Dumont GJ, de Visser SJ, Cohen AF, van Gerven JM. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. *Br J Clin Pharmacol*. 2005;59:495-510.
35. Staner L, Luthringer R, Macher JP. Effects of antidepressant drugs on sleep EEG in patients with major depression. Mechanisms and therapeutic implications. *CNS Drugs*. 1999;11:49-60.
36. Hassler G, Drevets W, Manji H, Charney D. Discovering major endophenotypes for major depression. *Neuropsychopsychopharmacology*. 2004;29:1765-1781.
37. Fu CH, Williams SC, Cleare AJ, et al. Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. *Arch Gen Psychiatry*. 2004;61:877-889.
38. Charney DS, Drevets WC. The neurobiological basis of anxiety disorders. In: Davis KL, Charney DS, Coyle JT, Nemeroff CB, eds. *Neuropsychopharmacology: the Fifth Generation of Progress*. Philadelphia, Pa: Lippincott Williams & Wilkins; 2002:901-930.
39. Hariri AR, Mattay VS, Tessitore A, et al. Serotonin transporter genetic variation and the response of the human amygdala. *Science*. 2002;297:400-403.
40. Hariri AR, Drabant EM, Munoz KE, et al. A susceptibility gene for affective disorders and the response of the human amygdala. *Arch Gen Psychiatry*. 2005;62:146-152.
41. Heinz A, Braus DF, Smolka MN, et al. Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nat Neurosci*. 2005;8:20-21.
42. Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM. Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol Psychiatry*. 2006 Feb 3;[Epub ahead of print].
43. Swerdlow NR, Koob GF. Dopamine, schizophrenia, mania, and depression: toward a unified hypothesis of cortico-striato-pallido-thalamic function. *Behav Brain Sci*. 1987;10:197-245.
44. Braff DL, Geyer MA. Sensorimotor gating and schizophrenia. Human and animal studies. *Arch Gen Psychiatry*. 1990;47:181-188.
45. Light GA, Braff DL. Human and animal studies of schizophrenia-related gating deficits. *Curr Psychiatry Rep*. 1999;1:31-40.
46. Siegel C, Waldo M, Mizner G, Adler LE, Freedman R. Deficits in sensory gating in schizophrenic patients and their relatives. Evidence obtained with auditory evoked responses. *Arch Gen Psychiatry*. 1984;41:607-612.
47. Boutros NN, Zouridakis G, Overall J. Replication and extension of P50 findings in schizophrenia. *Clin Electroencephalogr*. 1991;22:40-45.
48. Nagamoto HT, Adler LE, Hea RA, Griffith JM, McRae KA, Freedman R. Gating of auditory P50 in schizophrenics: unique effects of clozapine. *Biol Psychiatry.* 1996;40:181-188.
49. Adler LE, Olincy A, Cawthra EM, et al. Varied effects of atypical neuroleptics on P50 auditory gating in schizophrenia patients. *Am J Psychiatry*. 2004;161:1822-1828.
50. Chung C, Remington G. Predictors and markers of clozapine response. *Psychopharmacology*. 2005;179:317-335.
51. Mackeprang T, Kristiansen KT, Glenthoj BY. Effects of antipsychotics on prepulse inhibition of the startle response in drug-naïve schizophrenic patients. *Biol Psychiatry*. 2002;52:863-873.
52. Ludewig K, Geyer MA, Vollenweider FX. Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. *Biol Psychiatry*. 2003;54:121-128.
53. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*. 2001;156:117-154.
54. Oranje B, Van Oel CJ, Gispen-De Wied CC, Verbaten MN, Kahn RS. Effects of typical and atypical antipsychotics on the prepulse inhibition of the startle reflex in patients with schizophrenia. *J Clin Psychopharmacol*. 2002;22:359-365.

Free paper

Functional genomics in postmortem human brain: abnormalities in a DISC1 *molecular pathway in schizophrenia*

Barbara K. Lipska, PhD; Shruti N. Mitkus, PhD; Shiny V. Mathew, PhD; Robert Fatula; Thomas M. Hyde, MD, PhD; Daniel R. Weinberger, MD; Joel E. Kleinman, MD, PhD

*The disrupted in schizophrenia 1 (*DISC1*) gene has been identified as a schizophrenia susceptibility gene based on linkage and single nucleotide polymorphism (SNP) association studies and clinical data, suggesting that risk SNPs impact on hippocampal structure and function. We hypothesized that altered expression of* DISC1 *and/or its molecular partners (nuclear distribution element-like* [NUDEL]*, fasciculation and elongation protein zeta-1* [FEZ1]*, and lissencephaly 1* [LIS1]*) may underlie its pathogenic role in schizophrenia and explain its genetic association. We examined the expression of* DISC1 *and its binding partners in the hippocampus and dorsolateral prefrontal cortex of postmortem human brains of schizophrenic patients and controls. We found no difference in the expression of* DISC1 *mRNA in schizophrenia, and no association with previously identified risk SNPs. However, the expression of* NUDEL*,* FEZ1*, and* LIS1 *was significantly reduced in tissue from schizophrenic subjects, and the expression of each showed association with high-risk* DISC1 *polymorphisms. These data suggest involvement of genetically linked abnormalities in the* DISC1 *molecular pathway in the pathophysiology of schizophrenia.*

 Dialogues Clin Neurosci. 2006;8:353-357.

Schizophrenia is a syndrome characterized by psychotic symptoms (hallucinations, delusions, thought disorder, and cognitive impairment), with a prevalence approaching 1% worldwide. Schizophrenia is clearly a genetic disorder. Results from twin and adoption studies show a heritability estimate for schizophrenia of 70% to 90%.[1-3] However, analysis of recurrence risk estimates in families with one or more affected individuals clearly argues against schizophrenia being a single-gene disorder, even with the possibility of incomplete penetrance.[4] As in other psychiatric disorders, the mode of transmission for schizophrenia is complex and multifactorial, with the possibility of a number of genes conferring varying degrees of susceptibility. With this in mind, efforts have been directed at identifying allelic variants in genes that may confer increased risk for schizophrenia. Identification of schizophrenia susceptibility genes will also increase our understanding of the molecular pathways involved in the etiology of the disorder, and may offer new therapeutic targets.

DISC1 gene

The disrupted in schizophrenia 1 (*DISC1*) gene is a 414.3 kb gene located on chromosomal region 1q42.2, and consists of 13 exons. *DISC1* was originally identified as a candidate gene for schizophrenia in a large Scottish family, in

Keywords: *schizophrenia; gene; pathophysiology; functional genomics; prefrontal cortex; hippocampus; postmortem human brain*

Author affiliations: Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Md, USA

Address for correspondence: 10 Center Drive, Bldg 10, Rm 4N306, Bethesda, MD 20892-1385, USA
(e-mail:lipskab@intra.nimh.nih.gov)

which a balanced translocation involving chromosomes 1 and 11 was strongly linked to schizophrenia, schizoaffective disorder, bipolar affective disorder, and recurrent major depression.[5] In this family, carriers of the translocation were found to have reduced P300 amplitude, which is observed in some patients with schizophrenia.[6] Subsequent association studies identified numerous polymorphisms in the *DISC1* gene associated with schizophrenia and affective disorders, although different polymorphisms/haplotypes in various regions of the gene were implicated in these studies.[7-12]

In the adult mouse brain, *DISC1* is expressed widely, including in the olfactory bulb, cortex, hippocampus, hypothalamus, cerebellum, and brain stem. During development, DISC1 protein is detected at all stages, from embryonic day 10 (E10) to 6 months old, with two significant peaks of protein expression of one of the *DISC1* isoforms at E13.5 and postnatal day 35.[13] Interestingly, these time points correspond to periods of active neurogenesis and puberty in the mouse. These results suggest that *DISC1* may play a critical role in brain development, lending support to the neurodevelopmental hypothesis of schizophrenia.

DISC1 encodes an 854-amino acid (aa) protein, which shows no homology to other known proteins and little homology between species.[14-16] This amino-acid sequence predicts that the protein DISC1 may act as a scaffolding protein with multiple binding motifs, facilitating formation of protein complexes. The N-terminus (aa 1-347) contains nuclear localization signals, whereas the C-terminus (aa 348-854) appears to be important for microtubule and centrosomal targeting,[17-19] although no centrosomal localization has been detected so far for the native protein.

Although the precise function of DISC1 in the brain is unknown, a number of DISC1-interacting partners have been identified, including fasciculation and elongation protein zeta-1 (FEZ1), nuclear distribution element-like (NUDEL), and lissencephaly 1 (LIS1), which are known to play a role in neuronal development and functioning. Altered interactions between DISC1 and its binding partners are currently being investigated in order to understand more accurately the biology of DISC1 as a schizophrenia susceptibility gene.

DISC1 molecular pathway

In an effort to understand the cellular function of DISC1, yeast-two hybrid studies have been used to identify molecular interactors of DISC1. It was found that DISC1 has numerous binding partners, including NUDEL, FEZ1, activating transcription factor (ATF) 4/5, and microtubule-associated protein 1A (MAP1A).[15,17,18] NUDEL is a component of a pathway involved in cytoplasmic dynein movement, and is involved in neurofilament assembly, neuronal migration, and development of neurite morphology.[20-25] Overexpression of truncated DISC1 protein inhibits neurite outgrowth in PC12 cells, suggesting that the DISC1-NUDEL complex may be involved in neuronal outgrowth.[15,25,26] The hypothetical peptide product resulting from the Scottish translocation removes the interaction domain for NUDEL. The defective DISC1-NUDEL complex may be a cause of neurodevelopmental abnormalities in schizophrenia.[19] Recently, it has been shown that NUDEL oligopeptidase activity is under tight regulation through binding to DISC1, since a mutation very close to the DISC1-binding site of NUDEL abolishes this activity.[27] Interestingly, NUDEL cleaves a number of neuropeptides in vitro, some of which have previously been implicated in the pathophysiology of schizophrenia, including neurotensin (NT).[25,29] NT receptor agonists may be potential antipsychotics; thus, inhibition of NUDEL could lead to increase in local concentration of NT, which may have an antipsychotic effect.[27] Altered subcellular distribution of DISC1 has been reported in patients with psychosis and alcohol/substance abuse, with increased ratios of nuclear to cytoplasmic DISC1 protein levels in patients.[30] Cell culture studies in cortical neurons have found evidence that DISC1 may colocalize with mitochondrial markers, and that its subcellular targeting is independent of the NUDEL-binding site.[26] Hayashi et al[27] have also demonstrated that DISC1 and NUDEL bind in a neurodevelopmentally regulated manner and form a trimolecular complex with another protein, LIS1. LIS1 is involved in neuronal migration and corticogenesis. Although the function of this complex is currently unknown, it is thought to play a role in dynein-mediated motor transport.[27]

Another interacting partner of DISC1 is FEZ1, which is a mammalian homologue of the *Caenorhabditis elegans* UNC-76 protein, involved in axonal outgrowth and fasciculation. Miyoshi et al demonstrated that DISC1 participates in neurite extension through its C-terminal interaction with FEZ1.[31] The chromosomal location for *FEZ1* was previously implicated in a schizophrenia linkage analysis, although results from different populations

vary in significance.[32] A modest association between schizophrenia and *FEZ1* polymorphisms has been detected in a subset of Japanese patients.[33]

Abnormalities in a *DISC1* pathway in schizophrenia

In our laboratory, we have tested the hypothesis that altered expression of *DISC1*, and/or its molecular partners *NUDEL*, *FEZ1*, and *LIS1* may underlie its pathogenic role in schizophrenia and explain its genetic association.[34] We examined the expression of *DISC1* and these selected binding partners in postmortem human brain. We found no difference in the expression of *DISC1* mRNA in schizophrenia, and no association with previously identified risk SNPs (all F values <1.5, all P values >0.2). *DISC1* immunoreactivity was significantly, albeit modestly (by approximately 20%), increased in the hippocampus of patients with schizophrenia: $F(1,73)=3.6$, $P=0.05$. However, the expression of *NUDEL*, *FEZ1*, and *LIS1* mRNA was each significantly reduced in schizophrenic tissue in both the dorsolateral prefrontal cortex and hippocampus and the expression of each gene showed association with a high risk *DISC1* polymorphism (all P values <0.05).

These data implicate genetically linked abnormalities in the *DISC1* molecular pathway in the pathophysiology of schizophrenia. Given its role in brain development and plasticity via its interaction with a number of different proteins, *DISC1* remains a candidate gene for schizophrenia, and an understanding of its exact mechanistic role in neuronal pathways may shed more light on the disease.

Conclusions

Schizophrenia is a devastating neuropsychiatric disorder, the genetics of which has been under extensive investigation for several decades. Despite being an exceedingly complex disease in terms of both etiology and pathogenesis, recent research is finally shedding light on schizophrenia susceptibility genes. There are several genes implicated by association studies and postmortem findings. Prominent among them are the genes *COMT*, *DTNBP1*, *GRM3*, *DISC1*, *NRG1*, *AKT1*, *GAD1*, *RGS4*, and *DRD2*. *DISC1* and its binding partners *FEZ1*, *NUDEL*, and *LIS1* are involved in cytoplasmic dynein movement, neurofilament assembly, neuronal migration, and neurite morphology, and may play a role in the neurodevelopmental deficits observed in schizophrenia.

Although the precise neurobiological cause of schizophrenia continues to be unknown, the abundance of evidence regarding susceptibility genes for schizophrenia cannot be dismissed. Identification of the molecular and cellular mechanisms that link susceptibility genes to the neurobiological functioning of the brain continues to be a major focus of research. As evidence for the functioning of the various susceptibility genes increases, it may be determined that these genes operate in a convergent molecular pathway affecting neural development and synaptic plasticity. The disruption of multiple genes within this pathway may lead to the development of schizophrenia. Such a convergent biochemical pathway may also be an attractive target for therapeutic intervention. ❑

REFERENCES

1. Kendler KS. Overview: a current perspective on twin studies of schizophrenia. *Am J Psychiatry*. 1983;140:1413-1425.
2. Tsuang MT, Gilbertson MW, Faraone SV, et al. The genetics of schizophrenia. Current knowledge and future directions. *Schizophr Res*. 1991;4:157-171.
3. Kendler KS, McGuire M, Gruenberg AM, Walsh D. An epidemiologic, clinical, and family study of simple schizophrenia in County Roscommon, Ireland. *Am J Psychiatry*. 1994;151:27-34.
4. Risch N. Linkage strategies for genetically complex traits. II. The power of affected relative pairs. *Am J Hum Genet*. 1990;46:229-241.
5. St Clair D, Blackwood D, Muir W, et al. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet*. 1990;336:13-16.
6. Blackwood DH, Fordyce A, Walker MT, et al. Schizophrenia and affective disorders–cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *Am J Hum Genet*. 2001;69:428-433.
7. Devon RS, Anderson S, Teague PW, et al. Identification of polymorphisms within Disrupted in Schizophrenia 1 and Disrupted in Schizophrenia 2, and an investigation of their association with schizophrenia and bipolar affective disorder. *Psychiatr Genet*. 2001;11:71-78.
8. Ekelund J, Hovatta I, Parker A, et al. Chromosome 1 loci in Finnish schizophrenia families. *Hum Mol Genet*. 2001;10:1611-1617.
9. Ekelund J, Hennah W, Hiekkalinna T, et al. Replication of 1q42 linkage in Finnish schizophrenia pedigrees. *Mol Psychiatry*. 2004;9:1037-1041.
10. Hennah, W, Varilo T, Kestila M, et al. Haplotype transmission analysis provides evidence of association for *DISC1* to schizophrenia and suggests sex-dependent effects. *Hum Mol Genet*. 2003;12:3151-3159.

Genómica funcional en el cerebro humano postmortem: alteraciones de la vía molecular DISC1 *en la esquizofrenia*

Se ha identificado que el gen DISC1 (disrupted in schizophrenia 1) constituye una susceptibilidad genética para la esquizofrenia en base a datos provenientes de la clínica y de estudios de ligazón y de asociación de polimorfismo del nucleótido único (PNU), lo que sugiere que el riesgo de polimorfismos tendría su efecto en la estructura y función del hipocampo. Se ha propuesto la hipótesis que la alteración en la expresión del DISC1 *y/o sus moléculas asociadas* (NUDEL [nuclear distribution element-like], FEZ1 [fasciculation and elongation protein zeta-1] y LIS1 [lissencephaly 1]) *podrían tener un papel patogénico en la esquizofrenia y explicar su asociación genética. Se ha examinado la expresión de* DISC1 *y sus elementos de unión en el hipocampo y la corteza prefrontal dorsolateral de cerebros humanos postmortem de pacientes esquizofrénicos y controles. No se encontraron diferencias en la expresión del RNAm del gen* DISC1 *en la esquizofrenia, ni tampoco asociación con los PNUs de riesgo identificados previamente. Sin embargo, la expresión de* NUDEL, FEZ1 *y* LIS1 *se encontró significativamente reducida en tejidos de sujetos esquizofrénicos, y la expresión de cada una de ellas mostró asociación con polimorfismos de* DISC1 *de alto riesgo. Estos datos sugieren que en la fisiopatología de la esquizofrenia existe un compromiso genético en la vía molecular del* DISC1.

Génomique fonctionnelle dans le cerveau humain postmortem : anomalies d'une voie moléculaire DISC1 dans la schizophrénie

La découverte du gène DISC1 (disrupted in schizophrenia gene) *consiste en la mise en évidence d'une susceptibilité génétique fondée sur une étude de liaison et sur une étude du polymorphisme d'un gène (single nucleotide polymorphism, SNP). Les études d'association suggèrent que les mutations cliniques ont un impact sur la fonction et la structure de l'hippocampe. Nous avons émis l'hypothèse qu'une expression altérée du gène DISC1 et/ou de ses molécules associées (NUDEL,* [nuclear distribution element-like]*, FEZ1,* [fasciculation and elongation protein zeta-1]*, et LIS1,* [lissencephaly 1]*) pourrait jouer un rôle dans la pathogènie de la schizophrénie, expliquant ses associations génétiques. Nous avons étudié l'expression du gène DISC1 et de ses éléments de liaison dans l'hippocampe et dans le cortex préfrontal dorsolatéral de cerveaux humains* postmortem *de patients schizophrènes et de témoins. Nous n'avons trouvé aucune différence dans l'expression de l'ARNm du gène DISC1 dans la schizophrénie, et aucune association avec des mutations uniques (SNP) identifiées antérieurement. Cependant, l'expression des gènes* NUDEL, FEZ1 *et* LIS1 *était réduite de façon significative dans le tissu provenant des patients schizophrènes, l'expression de chacun de ces gènes montrant une association avec la mutation unique (SNP). Ces résultats montrent que dans la physiopathologie de la schizophrénie il existe des anomalies génétiques de la voie moléculaire du gène* DISC1.

11. Hodgkinson, CA, Goldman D, Jaeger J, et al. Disrupted in schizophrenia 1 (*DISC1*): association with schizophrenia, schizoaffective disorder, and bipolar disorder. *Am J Hum Genet*. 2004;75:862-872.

12. Callicott, JH, Straub, RE, Pezawas L, et al. Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. *Proc Natl Acad Sci U S A*. 2005;102:8627-8632.

13. Schurov IL, Handford EJ, Brandon NJ, Whiting BJ. Expression of disrupted in schizophrenia 1 (DISC1) protein in the adult and developing mouse brain indicates its role in neurodevelopment. *Mol Psychiatry*. 2004;9:1100-1110.

14. Ma L, Liu Y, Ky B, Shughrue PJ, Austin CP, Morris JA. Cloning and characterization of Disc1, the mouse ortholog of DISC1 (Disrupted-in-Schizophrenia 1). *Genomics*. 2002;80:662-672.

15. Ozeki Y, Tomoda T, Kleiderlein J, et al. Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. *Proc Natl Acad Sci U S A.* 2003;100:289-294.

16. Taylor MS, Devon RS, Millar JK, Porteous DJ. Evolutionary constraints on the Disrupted in Schizophrenia locus. *Genomics*. 2003;81:67-77.

17. Morris JA, Kandpal G, Ma L, Austin CP. DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. *Hum Mol Genet*. 2003;12:1591-1608.

18. Millar JK, James R, Brandon NJ, Thomson PA. *DISC1* and *DISC2*: discovering and dissecting molecular mechanisms underlying psychiatric illness. *Ann Med*. 2004;36:367-378.

19. Brandon NJ, Handford EJ, Schurov I, et al. Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. *Mol Cell Neurosci*. 2004;25:42-55.

20. Niethammer MD, Smith S, Ayala R, et al. NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron*. 2003;28:697-711.

21. Sasaki S, Shionoya A, Ishida M, Gambello MJ, et al. A LIS1/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. *Neuron*. 2000;28:681-696.
22. Sweeney KJ, Prokscha A, Eichele G. NudE-L, a novel Lis1-interacting protein, belongs to a family of vertebrate coiled-coil proteins. *Mech Dev*. 2001;101:21-33.
23. Feng Y, Walsh CA. Mitotic spindle regulation by Nde1 controls cerebral cortical size. *Neuron*. 2004;44:279-293.
24. Nguyen MD, Shu T, Sanada K, et al. A NUDEL-dependent mechanism of neurofilament assembly regulates the integrity of CNS neurons. *Nat Cell Biol.* 2004;6:595-608.
25. Shu T, Ayala R, Nguyen MD, Xie Z, Gleeson JG, Tsai LH. Ndel1 operates in a common pathway with LIS1 and cytoplasmic dynein to regulate cortical neuronal positioning. *Neuron*. 2004;44:263-277.
26. Brandon NJ, Schurov I, Camargo, LM, et al. Subcellular targeting of *DISC1* is dependent on a domain independent from the Nudel binding site. *Mol Cell Neurosci*. 2005;28:613-624.
27. Hayashi MA, Portaro FC, Bastos MF, et al. Inhibition of NUDEL (nuclear distribution element-like)-oligopeptidase activity by disrupted-in-schizophrenia 1. *Proc Natl Acad Sci U S A*. 2005;102:3828-3833.
28. Caceda R, Kinkead B, Nemeroff CB. Do neurotensin receptor agonists represent a novel class of antipsychotic drugs? *Semin Clin Neuropsychiatry*. 2003;8:94-108.
29. Kinkead B, Nemeroff CB. The effects of typical and atypical antipsychotic drugs on neurotensin-containing neurons in the central nervous system. *J Clin Psychiatry*. 1994;55(suppl B):30-32.
30. Sawamura N, Sawamura-Yamamoto T, Ozeki Y, Ross CA, Sawa A. A form of DISC1 enriched in nucleus: altered subcellular distribution in orbitofrontal cortex in psychosis and substance/alcohol abuse. *Proc Natl Acad Sci U S A*. 2005;102:1187-1192.
31. Miyoshi K, Honda A, Baba K, et al. Disrupted-In-Schizophrenia 1, a candidate gene for schizophrenia, participates in neurite outgrowth. *Mol Psychiatry*. 2003;8:685-694.
32. Lewis, CM, Levinson DF, Wise LH, DeLisi LE, Straub RE. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am J Hum Genet*. 2003;73:34-48.
33. Yamada K, Nakamura K, Minabe Y, et al. Association analysis of FEZ1 variants with schizophrenia in Japanese cohorts. *Biol Psychiatry*. 2004;56:683-690.
34. Lipska BK, Peters T, Halim N, et al. Expression of DISC1 binding partners is reduced in schizophrenia and associated with DISC1 SNPs. *Hum Mol Gen*. 2006;5:1245-1212.

Dialogues in clinical neuroscience

An interface between clinical neuropsychiatry and neuroscience, providing state-of-the-art information and original insights into relevant clinical, biological, and therapeutic aspects

1999
- Bipolar Disorders
- Depression in the Elderly
- Nosology and Nosography

2000
- Posttraumatic Stress Disorder
- Alzheimer's Disease
- From Research to Treatment in Clinical Neuroscience
- Schizophrenia: General Findings

2001
- Genetic Approach to Neuropsychiatric Disorders
- Schizophrenia: Specific Topics
- Cerebral Aging
- New Perspectives in Chronic Psychoses

2002
- Pathophysiology of Depression
- CNS Aspects of Reproductive Endocrinology
- Anxiety I
- Drug Development

2003
- Dementia
- Psychiatric Disorders in Somatic Medicine
- Anxiety II
- Chronobiology and Mood Disorders

2004
- Predictors of Response to Treatment in Neuropsychiatry
- Neuroplasticity
- Parkinson's Disease
- Mild Cognitive Impairment

2005
- Early Stages of Schizophrenia
- New Psychiatric Classification based on Endophenotypes
- Pharmacology of Mood Disorders
- Sleep Disorders, Neuropsychiatry, and Psychotropics

2006
- Diagnosis and Management of Schizophrenic Disorders
- Depression in Medicine

Back issues are available in pdf format on
www.dialogues-cns.org

Supported by an educational grant from Servier

Instructions for authors

AIM AND SCOPE

***Dialogues in Clinical Neuroscience* is a quarterly peer-reviewed publication that aims to serve as an interface between clinical neuropsychiatry and the neurosciences by providing state-of-the-art information and original insights into relevant clinical, biological, and therapeutic aspects. Each issue addresses a specific topic, and also publishes free contributions in the field of neuroscience as well as other non–topic-related material.**

GENERAL INSTRUCTIONS

Submission: Manuscripts may be submitted by e-mail (mail.dialneuro@fr.netgrs.com) or on diskette (3.5-inch, for IBM, IBM-compatible, or Apple computers), double-spaced, with 1-inch/2.5-cm margins. All pages should be numbered. All corresponding authors should supply a black-and-white portrait photograph for inclusion on the Contributors page at the beginning of the issue. This may be sent by e-mail, provided the resolution of the file is at least 300 dpi.

Title page: The title page should include a title, the full names of all the authors, the highest academic degrees of all authors (in country-of-origin language), affiliations (names of department[s] and institution[s] at the time the work was done), a short running title (no more than 50 letters and spaces), 5 to 10 keywords, the corresponding author's complete mailing address, telephone, fax, and e-mail, and acknowledgments.

Abstract: A 150-word abstract should be provided for all articles, except Posters. The editorial department will edit abstracts that are too short or too long. Abstracts will be translated into French and Spanish by the publisher's editorial department. Authors who are native French or Spanish speakers may choose to provide an abstract in their own language, as well as an English abstract.

Text: All texts should be submitted in English. Abbreviations should be used sparingly and expanded at first mention. A list of selected abbreviations and acronyms should be provided (or will be prepared by the editorial department) where necessary. The style of titles and subtitles should be consistent throughout the text. The editorial department reserves the right to add, modify, or delete headings if necessary. *Dialogues in Clinical Neuroscience* uses SI units and generic names of drugs.

REFERENCES

Citation in text: All references should be cited in the text and numbered consecutively using superscript Arabic numerals.

Reference list: Presentation of the references should be based on the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. *Ann Intern Med*. 1997;126:36-47 ("Vancouver style"). The author-date system of citation is not acceptable. "In press" references should be avoided. Titles of journals should be abbreviated according to *Index Medicus*. All authors should be listed for up to six authors; if there are more, only the first three should be listed, followed by "et al." Where necessary, references will be styled by the editorial department to *Dialogues in Clinical Neuroscience* copyediting requirements. Authors bear total responsibility for the accuracy and completeness of all references and for correct text citation.

Examples of style for references:

Journal article:

1. Heinssen RK, Cuthbert BN, Breiling J, Colpe LJ, Dolan-Sewell R. Overcoming barriers to research in early serious mental illness: issues for future collaboration. *Schizophr Bull*. 2003;29:737-745.

Article in a supplement:

2. Greenamyre JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment and mitochondria. *Parkinsonism Relat Disord*. 2003;9(suppl 2):S59-S64.

Chapter in a book:

3. Carpenter WT Jr, Buchanan RW. Domains of psychopathology relevant to the study of etiology and treatment in schizophrenia. In: Schulz SC, Tamminga CA, eds. *Schizophrenia: Scientific Progress*. New York, NY: Oxford University Press; 1989:13-22.

Web-based material:

4. Peripheral and Central Nervous System Advisory Committee. Meeting Documents. Available at: http://www.fda.gov/ohrms/dockets/ac/cder01.htm. Rockville, Md: Food and Drug Administration. Accessed October 21, 2004.

Presentation at a conference:

5. McGlashan TH, Zipursky RB, Perkins DO, et al. Olanzapine versus placebo for the schizophrenic prodrome: 1-year results. Paper presented at: 156th Annual Meeting of the American Psychiatric Association; May 17-22, 2003; San Francisco, Calif.

FIGURES AND TABLES

Figures should be of good quality or professionally prepared, with the proper orientation indicated when necessary (eg, "top" or "left"). As figures and graphs may need to be reduced or enlarged, all absolute values and statistics should be provided. Provide each table and figure on a separate sheet. Legends must be provided with all illustrations, including expansion of all abbreviations used (even if they are already defined in the text). All figures and tables should be numbered and cited in the text.

SPECIFIC FORMATS

Editorial: 400 words.

State of the art: 7000 words.

Pharmacological aspects; Clinical research; Basic research: 3000-5000 words.

Free papers: 2500 words.

Posters: 1500 words. These are usually two facing printed pages of the journal, and should include figures and/or images. Posters may need to be edited to meet formatting requirements. Legends must be provided with all illustrations.

EDITORIAL ASSESSMENT AND PROCESSING

Peer review: All contributions to *Dialogues in Clinical Neuroscience* will be reviewed by members of the Editorial Board and submitted to expert consultants for peer review. All contributions should be original review articles.

Editorial processing: All manuscripts are copyedited according to the guidelines of the latest edition of the *American Medical Association Manual of Style* (Baltimore, Md: Williams & Wilkins); the spelling used is American (reference dictionaries: latest editions of *Merriam-Webster's Collegiate Dictionary* and *Stedman's Medical Dictionary*).

Proofs: Page proofs will be sent to the corresponding author for approval in PDF format by e-mail. Authors who wish to receive a hard copy of their proofs should contact the editorial offices upon receipt of the proofs by e-mail. Author corrections should be returned within 48 hours by e-mail or fax. If this deadline is not met, the editorial department will assume that the author accepts the proofs as they stand. Authors are responsible for all statements made in their work, including changes made by the editorial department and authorized by the author.

COPYRIGHT

Transfer of copyright: Copyright of articles will be transferred to the publisher of *Dialogues in Clinical Neuroscience*. The Copyright Transfer Agreement must be signed by all authors and returned to the publisher.

Permissions: The author should inform the editorial office if any of the figures or tables are reproduced from elsewhere. For reproduction of copyrighted work, the editorial office will obtain authorization from the publisher concerned. Requests for permission to reproduce material published in *Dialogues in Clinical Neuroscience* should be sent directly to the editorial office (mail.dialneuro@fr.netgrs.com).