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PERSPECTIVES

Neuropsychopharmacology at the New Millennium: New Industry Directions

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Rapid advances in modern gene seeking techniques and the sequence data evolving from related genome research should provide both new targets for drug discovery and new insights into risk factors for many neurological and psychiatric disorders. Coupled with the high speed synthetic capabilities available in many companies, high-throughput screening is identifying potential novel drug candidates at extraordinary rates. This enables the drug discoverer to be more precise in the biological specificity of drugs taken to human trials thereby reducing the potential side-effect profile of clinical candidates. The ability to create large libraries of compounds also allows researchers to focus on metabolism and pharmacokinetics at an earlier stage in the drug development process to minimize drug-drug interactions via common sites of metabolism and optimize duration of action for particular indications. An emerging bottleneck in psychopharmacological drug discovery is the relative paucity of preclinical behavioral models predictive of clinical efficacy and the need to carry out early clinical trials to demonstrate therapeutic utility. However, through the use of recently developed chip technology, coupled with data bases of information about single nucleotide polymorphisms in potential candidate genes or risk factors for psychiatric disorders, it should be possible in the near future to stratify clinical populations genetically for inclusion in specific drug treatment trials. The ultimate goal of this research is to obtain homogeneous populations for trials and to predict risk before the phenotype of the disorder is manifest. ***[Neuropsychopharmacology 20:99–105, 1999]*** *© 1998 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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Leaving aside ethnopharmacology and traditional natural products, and the lore that surrounds them, neuropsychopharmacology is about 100 years old. Compared to the divergence of primate lines and the appearance of archaic Homo sapiens 500,000 years ago, or to the fringes of recorded history perhaps 15,000 years ago, the history of neuropsychopharmacology is a blink of the eye. However, in that 100 years (arguably the last 50) we have learned more about the human brain that largely evolved so long ago and how it functions (or fails to function) than we learned in the entire period preceding it. Technologies that have allowed these advances in the understanding of genes important in brain development and function and the discovery of novel therapeutics are evolving at rates that are difficult to encompass in traditionally formatted journals; I have therefore included two types of references in this article. The first is the traditional style reference to print literature while the second is to various web sites that may give detailed descriptions of the technologies as they are evolving. Hopefully, since many of these sites are continuously maintained, they will serve as up-to-date references that will evolve with the technology. Many of these sites are maintained by the companies that have evolved the technologies and so should be visited with the same critical judgement as evaluating a research article.

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REFINEMENT AND EXPANSION OF EXISTING THERAPEUTICS TARGETS

The careful examination of the molecular pharmacology of earlier generations of therapeutics has led since the 1950's to increased refinement of these drugs and has spurred two generations of research. The importance of dopamine as a transmitter important for schizophrenia and Parkinson's disease was recognized in the molecular pharmacology of chlorpromazine and l-dopa and generated at least two iterations of antipsychotic medication. Treatment possibilities with drugs like risperidone, olanzapine [http://www.zyprexa.com] and clozapine are possible because of these studies. Advocacy groups have also established web sites that are useful sources of information for patients and their relatives about the disorders and medications [http://www.nami.org]. The relationship of the actions of benzodiazepines to allosteric enhancement of GABA's action at its receptors has resulted in novel hyponotics like zolpidem [http://www.searlehealthnet.com] and new receptor subtype specific anxiolytics in development [http://www.nrgn.com]. Coupled with an increased appreciation of pharmacokinetics and half-life, these drugs are, or promise to be, improvements over the older generation of flurazepam, alprazolam, diazepam and other benzodiazepines in terms of side effects such as cognitive impairment and physical dependence. Tricyclic antidepressant pharmacology led to the discovery of the importance of serotonin in mood disorders, ultimately resulting in the newer and safer serotonin specific reuptake inhibitors for the expanded treatment of depression and related disorders [http://www.lilly.com; http://www.pfizer.com].

In parallel with the expansion of molecular pharmacological techniques over the last 20 years, molecular genetics has made a tremendous impact on the tools available to the molecular pharmacologist. Many of the enzymes and receptors for the drugs used in psychiatry have been cloned and, almost invariably, single receptors or enzymes were shown to be part of larger families with several homologous relatives. These closely related members of a family are subtly different in their distribution in the brain and their biochemical functions; differences in distribution and biochemistry may result in different behavioral actions mediated through the different subtypes. The indiscriminate action of a drug at a variety of subtypes of the same receptor may contribute to the side effect profile of the drug or work at cross purposes to the therapeutic effect. Five members of the dopamine receptor family (3 unknown until the era of cloning) and splice variants have been identified (Monsma and Civelli 1997). Drugs specific for each subtype are now available for both mechanistic studies and for consideration as a newer generation of therapeutics for schizophrenia, attention deficit disorder, substance abuse etc. (e.g. D4-antagonists; Tallman et al. 1997). Almost 20 separate subunits of GABA receptors are available for the potential combinatorial formation of thousands of pentameric GABA receptors; these also are being actively pursued as individual drug targets. A similar situation exists for the glutamate families of NMDA, metabotropic and AMPA receptors that have not been as fully explored as GABA. Potential new anxiolytic and antipsychotic drugs may come from the examination of agonists at specific subtypes of these glutamate receptors (Moghaddam and Adams 1998).

Taken together, over 1000 G-Protein Coupled Receptors (GPCRs), over 100 ligand-gated and other ion channel subunits, over 20 reuptake proteins, almost 50 cytokine, 25 nuclear receptor and several thousand other molecular targets have been cloned, expressed in mammalian, yeast, or insect cells, and are currently available to the pharmaceutical drug discoverer as potential therapeutic targets for agonist or antagonist discovery. Almost weekly, a new potential therapeutic approach to a known disorder is suggested by the proliferation of molecular or biochemical targets. Since in the previous 100 years therapeutics directed to 400–500 targets have been marketed by the pharmaceutical industry, this is an extraordinary position to be in. With several thousand potential targets (most of which are also found in the brain) available, target selection and validation is an important part of any research director's plan.

Target validation may take many forms. Relatedness to known drugs, transmitters or biologics of known function may suggest immediate applications. In some cases, the specific knockout of a gene or its hyperexpression in mice may indicate a potential function of the target; unfortunately, some knockout results have been confusing or actually misleading because of the redundancy of neural pathways. In other cases, identification of the localization of the prospective cloned target to a particular tissue or region of the brain may yield valuable information. A recent example where this approach was valuable was in the case of the hypocretin (orexin) peptides and receptors whose hypothalamic regional localization suggested involvement in eating (Lecea et al. 1998). In a similar manner, the positional cloning of the gene coding for the leptin peptide from ob/ob mice and its subsequent localization to adipocytes identified it as the long sought for lipostatic factor (Zhang et al. 1994) [http://www.amgen.com]. The identification of leptin receptors in relevant hypothalamic regions confirmed this hypothesis. The subsequent localization of the same leptin receptors on other cells suggested a more general trophic function for this peptide (Tartaglia 1997) [http://www.mlnm.com]. Similarly the localization of genes in the nervous system coding for proteins, called neuroimmunophilins, similar to the binding site for immune suppressants like Cyclosporin A suggest utility of drugs like FK-506 in

neuroimmunology. The further interaction of receptors with calcineurin suggest the potential for novel therapeutics related to FK-506 for neurodegenerative disorders (Snyder et al. 1998) [http://www.guilfordpharm.com]. At a more genetic level, the positional cloning of the gene mutated in Werner's syndrome, an autosomal recessive disorder characterized by premature aging, resulted in the identification of a DNA helicase as the gene product of the mutated site (Yu et al. 1996). This finding provided the first insight into specific biochemical factors involved in the aging process and raise the interesting question of whether helicase inhibitors might enhance the rate of aging in patients treated with them and the extremely unlikely possibility that a therapeutic activator of this enzyme might slow the aging process. With the extensive focus on identification of expressed sequences through the analyses of various databases [http://www.incyte.com; http://www.ncbi.nlm.nih.gov; http://www.tigr.org; http://www.hgsi.com], one might anticipate that early in the next millennium most expressed sequences will be identified at least as orphan targets of unknown function. Their relationship to existing targets and localization may suggest function and research directed to this end should continue to be important for the forseeable future.

Combinatorial Chemistry - A Toolbox for Discovery

Combinatorial chemistry has revolutionized the way that pharmaceutical investigators look for novel drug candidates. The origins of this approach date to about 35 years ago where scientists interested in peptide and nucleic acid research began to develop methods to synthesize small peptides and oligonucleotides by hand. While initially tedious and time consuming, the chemistries that were developed were robust enough in yield and suitable for automation. The availability of an ever increasing need (market) for these reagents resulted in automated peptide and oligonucleotide synthesis by the early 1980's [Applied Biosystems now part of http://www.perkin-elmer.com]. The availability of these tools led early to the elaboration of antisense strategies that have recently resulted in the approval of a novel antisense therapeutic (fomivirsen) for the treatment of cytomegalovirus induced retinitis in AIDS patients [http://www.isisph.com]. Further method development led to the formation of combinatorial libraries of peptides and oligonucleotides of various size and enormous combinatorial diversity, including short peptides on chips [Affymax now part of http://www.glaxo wellcome.com]. Similar approaches were applied to carbohydrates to form additional libraries and diversity. The use of these libraries led to the development of higher throughput screening methodologies (described below) and the identification of potentially useful leads for enzyme and receptors targets in addition to the more obvious use of antisense drugs as transcriptional regulators.

While in vitro hits in biochemical assays were attainable with these methodologies, peptides, oligosaccharides and polynucleotides are generally not very useful as orally active therapeutics and, in particular, therapeutics for CNS applications. One goal of combinatorial research became to reduce the size of the oligomer to 3–5 units and the minimal structure to obtain significant biological activity (affymax technology). By modeling these smaller structures, the investigators attempted to develop a pharmacophore hypothesis to allow the rationalization of a small molecule discovery program. Because of the enormous conformational flexibility of even smaller oligomers, this proved to be an intractable problem, not solvable with current technology. The relationship of the enkephalins to morphine can be made post hoc but the synthesis of morphine would not be immediately predicted based upon the enkephalin peptide structure. Replacement of peptide bonds with more stable linkages led to "peptoids" which provided greater metabolic stability but did not aid in bioavailability, particularly in the central nervous system. Thus the stage was set for the application of combinatorial methodology to more conventional drug-like molecules.

Privileged Structures–Conserved Binding Motifs

Over the last 50 years, it has been long recognized by medicinal chemists that there are certain heterocyclic structures (called privileged structures or conserved binding motifs) that have been useful for the development of therapeutics (Evans et al. 1988; see also Tallman and Dahl 1995). In addition, as molecular pharmacology became more sophisticated, the interaction of drugs with multiple receptor subtypes pointed to a similar pharmacophore or binding pocket present in apparently unrelated receptors. The older generation of antipsychotics are particularly prone to interaction with muscarinic cholinergic, serotonin, adrenergic, dopaminergic, histamine and probably a variety of peptide receptors. This suggested a similar binding pocket on many of these receptors. What was not appreciated until recently was the relatedness of these seemingly diverse receptors at a molecular level and the strong sequence homology of the G-protein coupled receptor (GPCR) family (to which all these receptors belong) in the trans-membrane spanning regions. Mutagenesis of individual members of the GCPR points frequently to the interaction of small molecular weight heterocyclic drugs with these regions (Wess 1997). The appreciation of this relationship has provided a convergence of receptor molecular structure with empirically-based me-

dicinal chemistry and a library strategy for approaching many members of the GPCR–even where drugs, i.e. "prior art", that interact with these receptors do not exist.

Among the tools that developed to exploit the concept of conserved binding motifs were various library construction paradigms. One paradigm focused upon the 1,4-benzodiazepine nucleus as a "privileged" motif (Evans et al. 1988; Brunin et al. 1996). Methods were developed to couple this nucleus to a solid matrix. Different but related benzodiazepines containing functional groups were coupled with a diverse set of different reagents rapidly creating a diverse set of compounds with a common core. The compounds could then be released from the support, isolated, and used for screening. A variant of this technology makes use of more random synthesis on beads in a batchwise fashion (Ni et al. 1996) [http://www.pharmacopeia.com]. Each bead has a small molecular weight identifier that can be used to reisolate the bead and identify the compound synthesized on the bead. This technique allows more random synthesis to occur and greater diversity to be obtained in a batchwise fashion.

While solid matrix strategies have dominated the combinatorial area, only a limited number of chemistries are amenable to solid state synthesis. Liquid synthetic stations have allowed the rapid analoging around particular structures of interest and the formation of more interesting screening libraries than the solid state methodologies. The technical aspects of synthesis and quality control for robotic work stations of this sort have been perfected and make use of sophisticated computerized controls [http://www.nrgn.com/AIDD]. Search strategies for the compounds in the real library and virtual libraries (composed of the individual fragments of the real libraries linked in every possible fashion) have become routine and fast. Virtual fragment space can be searched after biological screening by forming neural network-derived data sets of predicted data based upon empirical training sets. This allows prioritization of libraries of 10^9 or more members. Thus, through a series of iterative screening cycles with the progressive refinement of models, compounds with very high affinity for the target can be discovered in a cost efficient manner.

High Throughput Screening and Reporter Technologies

Most academic investigators are familiar with the traditional 96-well plate format where the wells are arranged in an 8×12 array and contain several hundred microliters of sample. These plates have become a standard for tissue culture and immunological experiments and are frequently used in receptor assays. Many current plate readers and scintillation counters are able to handle this format and today it allows fairly rapid and reliable assay. Multiple well pipettors and liquid handling devices allow a relatively high throughput rate of assays to be carried out. Coupled with robotic arms dedicated to the movement and warehousing of these plates, most organizations can carry out screening in a rapid and efficient fashion. One of the concerns in the industry has been the perceived need to screen all of the large number of compounds produced by combinatorial chemistry. While I believe that computer-assisted screening based upon sucessively more precise pharmacophore hypotheses can be used to solve problems in a cost efficient manner, others see the need to screen every compound. This may indeed be the case where a diversity library is sampled for a novel drug target but the costs of this random screening may be prohibitively high. To circumvent these prohibitively high costs, miniaturization of screening for volumes as small as a single microliter have been proposed along with an increase of the number of assays per plate of 3456 [http://www.aurorabio.com]. This allows 36 times the number of assays to be carried out with reduced amounts of reagent.

To do this there has been a need to develop fluorescent-based assays in substitute for radioactivity-based screening. These assays may be based upon fluorescence energy transfer reactions between two fluorescent groups located on separate proteins either enhanced or reduced by a drug or through the use of reporter enzyme constructs; two major such enzymes are luciferase and beta-lactamase. Coupled with the correct transcriptional regulators and promotors, these enzymes can be induced by a diverse series of targets that activate transcription, including G-protein coupled receptors. Theoretically, it is possible to search for either agonists or antagonists of a receptors in this manner or for drugs that might activate or inhibit transcription directly. Blue/green fluorescence markers can indicate the presence or absence of activity in single cells or incubations in response to various forms of stimulation through the hydrolysis of fluorescent substrate by the induced enzyme (Zlokarnik et al. 1998). Other laser based fluorescent technology can look at the relative distribution of voltage sensitive dyes and Ca++ imaging in small groups of cells, currently in 96-well format [FLIPR technology Molecular Devices]. While space precludes a detailed description of all possible variations, enzymes, protein-protein interactions, transcriptional controllers and many other biological interactions can be efficiently assayed in this fashion. Screening in novel ways frequently requires the rethinking of our conventional slower methods of the past. For example, second messenger assays or electrophysiological approaches are amenable to the detailed investigation of the behavior of single compounds, not millions. These have become secondary assays and are now more useful at later stages in the discovery process.

Genomics and its Potential Impact on Pharmaceutical Discovery

Beyond what has already been described from the identification of the familial relationships of receptors and other targets, the impact of molecular genetics has been felt in every corner of biology and clinical medicine. This impact, primarily the result of the last ten years work, will have repercussions into the next millennium. A number of technical developments in high speed robotic sequencing and data handling have occurred in parallel to those used in high-throughput screening described earlier. In the next ten years, the interactive force of these developments will change the way we view the genetics of psychiatric disorders and develop therapeutics for these disorders.

The Human Genomic project [http://www.nhgri.nih.gov] (not quite a decade old) hopes to obtain the DNA sequence of the entire array of chromosomes by the year 2005. A more aggressive three year schedule has been proposed by a private organization [http://www.celera.com]. This gigantic enterprise—the biological equivalent of landing on the moon—has been made possible through the enormous technical strides in high-throughout sequencing and data handling developed both in the government [http://www.ncbi.nlm.nih.gov] and in private institutes [http://www.tigr.org] and "for profit" companies [http://www.hgsi.com; http://www.incyte.com]. Perhaps even more important than knowing the linear sequence of nucleotides that make up the DNA of a particular individual is the roadmap to genes of interest that has been provided by the identification of expressed sequences [tigr; incyte; hgsi]. These expressed sequences give a window into the particular proteins and the transcription factors and regulation characteristic of an individual tissue. Perhaps half of the 100,000 or so individual genes that are present in humans are expressed in the brain itself and the regional distribution of these genes is still an unstudied area. While vast areas of the genome have no known function, hot spots of DNA are being identified throught the use of expressed sequence tags (ESTs); these areas frequently contain many unrelated genes.

As an example of one such hot spot, the deletion of 2M bases by unequal cross-over at a recently duplicated gene (Jurado et al. 1998) results in William's syndrome with a clinical phenotype that contains not only characteristic facial features but also cardiovascular changes in affected patients; of greater interest to readers of this review are the particular neurological signs found in patients with this disorder. This particular defect at chromosomal location 7q11.23 results in a disorder characterized by the above changes and also with neurological differences including enhanced sociability/linguistic/musical skills coupled with diminished spatial/mathematical ability (Anonymous editorial 1996). There is a very good and informative site for those interested in William's syndrome [http://www.wsf.org]. Current YAC technology (yeast artificial chromosome) allows these regions of the chromosome to be identified, isolated and sequenced. The sequence then can be used with data base information to identify particular genes that are deleted. In the case of William's syndrome where some of these and related techniques are being applied, the deletion of this region is due to unequal crossover at two recently duplicated genes allowing for mismatch. The consequences are the hemizygous deletion of the gene for elastin important in facial structure and cardiovascular function and one of the two normal copies of several unrelated structural and functional genes probably involved in human neural development (e.g. the human homolog of the Drosophila frizzled wnt receptor gene (Wang et al. 1997)). Individuals with this disorder are heterozygously deleted at this site; since a parallel gain of function mutation with extra gene copies might be predicted to be equally likely, it is interesting to speculate what phenotype extra copies of these genes might cause in humans. A tremendous amount of work has gone into these investigations and while by no means diminishing this work, in 10 years time the linkage markers throughout the genome will be known and will allow rapid positional identification of these rare chromosome deletions and of the functions of genes found in the deleted areas. With the large number of genetically-based developmental disorders (Epstein 1995), and additional subtle disorders of heterozygotes, this type of analysis should redefine many disorder classifications and may suggest potential early stage therapeutic intervention.

Many of these specific deletion disorders are quite rare. In contrast, schizophrenia, mood disorders, dementias, and anxiety affect significant percentages of the population. Thus, they show potentially more in common with the genetics of obesity, essential hypertension, diabetes and other common conditions than with the genetics of simple Mendelian disorders (Weissman 1995). Many who are concerned with these common disorders think about "risk factors" that interact with environmental factors resulting in the expression of the disease phenotype (Weber 1996). The evolving literature in Alzheimer's disease points to a number of risk factors for both genetic and "sporatic" forms and early and late forms. No predictive test is available and a single test may never be predictive; however, positive tests of several factors may increase the probability (risk) of developing the disorder [http://www.alz.org; http://www.alzheimers.org.uk; an individually maintained research-oriented site http://dsmallpcz.path.unimelb.edu.au/ad.html]. Similar thinking is current in the area of schizophrenia which is thought of as a developmental disorder with potential genetic, infectious and environmental factors interacting to cause manifestations of the

disorder late in life. While single dominant risk genes in schizophrenia are unlikely, it is possible that an accumulation of several slightly deficient normal alleles may contribute to the risk of schizophrenia under the right environmental circumstances. This situation might be compared to the role that normal allelic variants of angiotensinogen may contribute to essential hypertension (Lifton 1996). There is a need to characterize the polymorphic behavior of candidate genes in major psychiatric disorders and the variation in some of the weakly linked sites on various chromosomes to the expression of the disease.

Single nucleotide polymorphisms (SNPs) can be detected via chip technology where fine genetic analysis is possible using specific oligonucleotide etched onto and synthesized on small glass chips [http://affymetrix.com] (Fodor 1997; Cho et al. 1998). Matches and mismatches can be used to genotype an individual (Cohen 1997a,b). The ultimate use of this data might be to genotype an individual, identify isogenes or predict response to a particular drug or therapeutic regimen [http://www.genaissance.com]. Similarly, grouping individuals according to their genotype may provide a useful inclusion/exclusion criteria for clinical trials. This rapidly developing area is called pharmacogenomics and in the next ten years will impact clinical trials in psychiatry and other medical areas (Weber 1996). At a more general level and perhaps more attainably, even drug metabolism and potential lethal drug interactions can be predicted by examining polymorphisms in cytochrome P-450 enzymes (2D6 for example).

Another related use for the chip technology is to examine using microarray techniques the patterns of mRNA (really the cDNA prepared from RNA samples) formation either as a result of disease (eg. human cancer (Derisi et al. 1996)) or as a result of drug treatment [synteny technology in http://www.incyte.com; http://affymetrix.com]. Through subtractive methods, unique genes formed or turned off by the manipulation (disease, drug, behavior, stage of development) can be identified, sequenced and compared to existing structures in the large number of EST databases (Wang et al. 1996). Interesting novel genes or potential therapeutic targets can thus be identified, put into screening, or used as therapeutic end points. The systematic change in gene arrays as a result of continued drug therapy could potentially be a surrogate endpoint for successful treatment. Much work still needs to be done in this area to make these techniques work quantitatively and not just qualitatively but this will be an emerging area of the next decade.

CONCLUSIONS

Over the next 25–50 years the amount of information available about neuronal function, circuitry and development will increase exponentially. There will be a progressive evolution to electronic information and on-line publishing of this information. Many more details will be known about individuals, their genetic structure, medical history and susceptibilities to various disorders. I view this information revolution which is just over the horizon with a great deal of caution. All of us are undoubtably at risk for or carriers of several lethal disorders; ethical and privacy issues about how this information can, should and will be used by organizations that control health care in this country are perhaps more important than the science. I hope that these issues will be more debated than the objective scientific data.

Although one can hope for a miracle, it is also likely that our brains will not evolve several new cortical layers to encompass the information-rich environment in which we will find ourselves during this period when compared to the original environment and time scale in which modern humans evolved. At best, we can anticipate the further refinement of existing therapies and the emergence of entirely new agents, insights into the etiology of neurological and psychiatric disorders, and methods of treatment. They will continue to be needed.

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