GENES, BEHAVIOUR AND HEALTH

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During the course of the 19th and 20th centuries psychiatry separated itself from neurology and focused on descriptive and psychosocial studies of so-called “functional” brain disorders, including behavioural disorders, while neurology encompassed diseases for which an organic substrate could be identified or readily postulated. The advent of the revolution in molecular genetics in the last two decades has led to the discovery of the genetic basis of several neurological diseases with important behavioural components, including such conditions as Huntington’s disease and Parkinson’s disease. This success in the genetics of neurological disorders has given investigators the hope that the same methods can unravel the behavioural abnormalities of psychiatric disorders. However, in spite of considerable work there has been to date no uncontested genetic finding in psychiatry.

Possible reasons for such an apparent lack of success include the following. First, it is possible that findings have not yet emerged; however, research efforts should continue to explore functional mutations that may contribute to the pathogenesis of major psychiatric disorders, particularly as an increasingly high number of genes is identified through the Human Genome Project. Alternatively, it is possible that psychiatric disorders, which are syndromically defined, will turn out to be highly heterogeneous in their genetics. Thus, conventional approaches that group large numbers of patients would not be likely to succeed if a variety of genetic mutations or polymorphisms may be present in such large populations. A scientifically more daunting scenario would be presented by causation or susceptibility due to multiple quantitative trait loci. According to such a hypothesis, predisposition to a common, complex behavioural disorder, such as depression, may be due to the the combination of multiple genes of small effect, none of which would in isolation be necessary or sufficient to cause the disorder. Thus, major depression would occur against a background where genetically susceptible individuals would be exposed to environmental (psychosocial)
stressors. In such a scenario there might be, for example, 50 genes of small effect; susceptibility might occur in the context of specific genetic variations in 10 or more of those genes. Such a complex situation would have been in the past virtually impossible to unravel. However, recent developments in sequencing methods, the availability of DNA chips, and highly sophisticated bioinformatics will permit the simultaneous assessment of hundreds or thousands of single nucleotide polymorphisms and the analysis of such complex data sets will be greatly facilitated by high-speed computing.

New methods for the behavioural analysis of animals with knockouts or overexpression of specific genes, as well as gene transfer methods to adult individuals, may further facilitate understanding of the behavioural effects of specific genes that are discretely expressed in specific brain locations.

An additional level of complexity, particularly in the areas of brain functioning and behaviour, is provided by the phenomenon of epistasis. Epistasis is defined as the influence of the genotype at one locus on the effect of a mutation at another locus; it plays a crucial role in a variety of evolutionary phenomena such as speciation, population bottlenecks, and possibly the varied repertoire of adaptive behavioural responses. Thus, clinical heterogeneity, multiple quantitative trait loci of small effect, and epistasis may be roadblocks hindering the immediate genetic dissection of behaviour and its disorders. Nevertheless, the rapid rate of progress in the fields of molecular biology, genetics, and informatics should soon overcome these difficulties and lead to a better understanding of the role of genes in behaviour, health and disease.

C. Liana Bolis and Julio Licinio
THE INTERFACE BETWEEN NEUROLOGY AND PSYCHIATRY

C.L. Bolis

INTRODUCTION
Today neurobiological and psychiatric conditions may be studied in a new and holistic way, and the concept of integrated clinical neuroscience has emerged. The brain has been recognized as correlated to peripheral and behavioural functions since ancient times; however, detailed understanding of its integrating activities has been reached only in recent times.

The scientific discoveries starting in the 20th century, in particular studies of the cellular membrane, myelin, the structure of the nerve and nerve excitation, synapses, axonal transport, neurotransmitters, neurohormones and neuromodulators, provided clues on basic neuronal activities underlying integrated physiological functions (motor and sensory behaviour) (Bolis, 1997).

Within this context, a multidisciplinary approach, extending from molecular to basic and applied science, has become increasingly important, making use of anatomy and microanatomy, biochemistry, biophysics, neuroimmunology, neuroendocrinology, physiology, neuroimaging, genetics, and anthropology. At the same time, however, during this transitional period due to the volcanic eruption of scientific information an ever-wider division became apparent between neurology and psychiatry. Neurology was perceived as more directed to the study of motor and sensory disturbances, i.e. more organic and testable dysfunctions, as opposed to psychiatry, which focused more on emotional behaviours, difficult to test and to correlate to an organic function/dysfunction. Over a long period, psychiatry, a medical discipline, took a more “humanistic” approach, at the frontiers of psychology, anthropology and philosophy.

The artificial separation of neurology and psychiatry is evident from the fact that some neurological disorders clearly have psychiatric components, as in the case of the well-known complex Parkinson-dementia of Guam and Creutzfeldt-Jakob disease and related pathologies.
Two factors contributed to the dichotomy that started in the last century between psychiatry and neurology. The first factor was that for some diseases of the central nervous system (CNS) classified as tumoral, vascular, infectious, and neurodegenerative, a biological substrate could be identified in the brain, whereas for other disorders that include schizophrenia, anxiety, depression and dysthymia a biological substrate could not be identified by traditional methods of study. At the same time the emergence of psychoanalysis led to theoretical models for disorders based on early life experiences and unconscious factors. As the 20th century comes to an end, a revolution in research methods in the basic neurosciences has resulted in an intensive search for fundamental biological mechanisms in both psychiatric and neurological disorders. The techniques of molecular biology, molecular genetics, cellular biology, imaging and increasingly sophisticated studies of neurotransmitters and neurohormones have made the barrier between psychiatry and neurology once more artificial and increasingly irrelevant to the understanding of the biological bases of brain disorders.

NEUROLOGY VERSUS PSYCHIATRY: A HISTORICAL PERSPECTIVE

In the past, observations were based on gross anatomy and on the study of the effect of traumatic events involving the head, as illustrated in an Egyptian papyrus found at the beginning of this century and dated 3000 years B.C. In principle, the association proposed between head injuries and loss of function was correct, but we are only now beginning to understand the mechanisms underlying physiopathological conditions and the relation between brain-mind and body.

Recent developments related to the genetic basis of behaviour are leading to the emerging concept of “clinical neuroscience”, which applies to all diseases having the brain as their biological substrate. This conceptual evolution is leading to new epistemological definitions that bring together “psychiatric disorders” and “neurological conditions” as organically based, neuroanatomically, and genetically determined, or carrying genetic predisposition, dysfunctions of the central nervous system (CNS). During the course of medical history, clinical observation and practice did not specifically differentiate conditions that we now call neurological from those currently classified as psychiatric. The historical concept of psychiatry as a branch distinct from neurology is relatively recent and dates from the late 19th century. During the middle ages some neurological syndromes were seen as “medical,” such as gait disorders, and received medical attention, although not effective cure. In contrast, disorders of the brain, such as epilepsy, hysteria, schizophrenia and depression, all belonged to the concept of insanity.
For most of medical history, however, the distinction between neurological and psychiatric disorders was very tenuous. For instance, Thomas Sydenham described the chorea now known by his name in 1686, but called it St. Vitus dance, referring to the patron saint of epileptics and those who are mentally insane. The same disease was also known as tarantism in southern Italy, as it was thought to be caused by the bite of the tarantula spider. Today, we can confirm that Sydenham may not have been very far from the truth. Recent studies have suggested that Sydenham chorea may have an autoimmune component, resulting from infection with group A β haemolytic streptococcus (Swedo et al., 1998).

In the second half of the 19th century positivistic theories led to the idea that everything could be scientifically explained, and researchers devoted their interest to many diseases, including paralysis, choreas, tremors, muscular disorders and also epilepsy whose clinical manifestations could be clearly identified and recorded as following specific patterns, and in many cases being directly or indirectly related to brain pathology that was visible with the aid of the scientific armamentarium of that time. Moreover, disorders with similar characteristics could also be observed in animals, resulting in valuable animal models that greatly facilitated the advancement of scientific knowledge. What was classified as psychiatric disorders (insanity) were those conditions that lacked a specific biological substrate or animal model. The lack of an organic basis that could be detected by the tools of the 19th and early 20th century led physicians and scientists to define psychiatric disorders as “functional”, and neurological disorders as “organic”.

In addition to the very incomplete understanding of the biology of psychiatric disorders, other important considerations had to be taken into account in the management of behaviourally disturbed individuals in large, densely populated urban areas in the last 100 years. For most of that time the severely mentally ill were seen as a social danger, and were therefore confined in asylums and subjected to laws that considered them as felons. Over the course of the last 50 years progress in the treatment of mental illnesses and further understanding of their neurobiological basis has led to considerable improvement in the medical and social treatment of the mentally ill and to a rapprochement between psychiatry and neurology.

Progress in the genetics of neurological disorders is now guiding the development of a neuroscience-based psychiatry that is fully integrated with neurology and basic neuroscience. In 1872 George Huntington comprehensively described a disease characterized by chorea and dementia, first recognized by Waters in 1842, and showed that it is a progressive hereditary disorder. Now we know that the Huntington
disease gene, encoding for a protein called “huntingtin”, is located near the tip of the short arm of chromosome 4. In contrast, such progress has not been achieved with other conditions. During the same period Charcot in France started studying women with hysteria and treating them using hypnosis. He carefully described the functional and behavioural alterations in his patients (suggestive pictures taken during his lessons can be found in every textbook of psychiatry), and as hypnosis appeared to be successful in most of the cases, he suggested that the cause of the disorder might be located deep in the brain (Owen, 1971). However, the CNS substrates of hysteria remain unknown.

At the dawn of the 20th century philosophical thought in Germany devoted much attention to the place of the individual within the context of existence as a whole, disregarding religious ideas of sin and punishment and emphasizing the uniqueness and isolation of the individual experience in a hostile or indifferent world. According to these concepts, any individual has freedom of choice and is responsible for the consequences of his or her actions. Existentialism, whose first advocate was Brentano in the 1880s and whose major exponent was Martin Heidegger (Heidegger and Brock, 1949), and phenomenology, a movement originated by Husserl in 1905 (Husserl, 1969) that considers human awareness in the light of objective reality or subjective response, strongly influenced the first attempts at classifying mental disorders by Kraepelin and Bleuler. Although Kraepelin’s phenomenology can be considered the basis for today’s most-used manual of mental diseases, DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994), it was a naturalistic description of symptoms characteristics of various disorders, but the possible etiology was completely elusive at the time, and so remained for decades until today.

Sigmund Freud started his research as a neurologist influenced by Charcot and his hypnosis treatment, but he soon discovered that in many cases hypnosis was not effective. As any possible biological explanation appeared to be inapplicable to his patients, he developed his psychodynamic theory based on the concepts of the conscious, subconscious and unconscious (ego, super-ego and id). Such concepts lacked a biological basis; furthermore, Freud preferred not to classify patients’ illnesses systematically using rigorous phenomenological descriptions (Freud, 1920). As the understanding that early life events influence vulnerability to mental illness became increasingly accepted, the division between neurological disorders and psychiatric disorders broadened, and epistemologically psychiatry moved away from medicine towards the humanities, conceptually approaching disciplines such as philosophy and the social sciences.
This reinforced the conceptual distinction between neurology, which dealt with diseases whose etiology was progressively better understood, and psychiatry, which dealt with functional disorders that lacked any biologically evident basis. There were no bridges across the boundaries between neurology and psychiatry for many years, until recently, when the success of the application of neuroscience and molecular genetics to neurology led the way for psychiatry to follow the progress achieved in neurology and to seek cellular, molecular, and genetic substrates for psychiatric disorders also, thereby incorporating their study into what we now call “clinical neurosciences”.

PHARMACOLOGY

The first stimulus to reduce resistance to the concept of organic biological bases for behaviour and mental illness was the discovery and evolution of pharmacological treatment of mental disorders. In the late 1950s there was a burst of serendipitous discovery of effective psychotropic drugs that included neuroleptics, antidepressants, the anxiolytic benzodiazepines and lithium. The discovery and understanding of the profile of action of these drugs on brain neurochemistry, jointly with the unequivocal evidence for their clinical activity, forced scientists to assume that certain abnormal behaviours could be controlled by acting on specific brain targets. This was already known and accepted in the case of neurological diseases.

For a long time the diagnostic characteristics of anatomopathological disorders were not evident in the case of psychiatric disorders. Nevertheless, it was recognized that pharmacological therapy using antidepressants was effective. Indeed it is well known that norepinephrine, the target of many antidepressants, is particularly important in brain metabolism, indicating that the antidepressant drugs, studied by Axelrod (Axelrod et al., 1961), interact with this representative of brain activity, directly related to metabolism. This was crucial to visualizing some psychiatric disorders such as affective disorders and schizophrenia as the consequence of deregulation of some biochemical processes.

These studies were also fundamental in the implementation of research related to neuroendocrinology and the involvement of this system in the physiology of cortical and limbic neuronal circuitry. They provided the basis for improving the activity of neuroleptics with a much lower risk of causing acute extrapyramidal side-effects.

It is expected that increasing knowledge of brain biochemistry and physiology in neurological and psychiatric sciences will contribute to the development, based on clinical evidence, of a series of new drugs for specific targets.
GENETICS
Recent progress in genetics has integrated many concepts which had previously appeared to be disparate and has further bridged the gap between the study of neurological and psychiatric conditions. For example, Gilles de la Tourette syndrome has been described as tics that range from intermittent simple brief jerks to a complex pattern of rapid, coordinated, involuntary movements. This disorder is in most cases associated in childhood with obsessive-compulsive disorder, once a 'truly' psychiatric illness, characterized by obsessions, i.e. thoughts that do not make any sense and keep recurring even when the subject tries not to have them, and/or compulsions, i.e. repetitive behaviours such as hand-washing (Leonard et al., 1992). Tourette syndrome appears to be inherited with autosomal dominant transmission (Eapen et al., 1993). Recently symptoms of exacerbation have been found to be related to streptococcal infection in early infancy (Swedo et al., 1998).

A key conceptual advance that has been brought from neurology into the realm of psychiatric disorders is the phenomenon described as "anticipation", which defines an earlier age of onset within succeeding generations of affected individuals. That concept was rejected in the past as genetically implausible, and it is now known to result from a progressive expansion in successive generations of nucleotide triplets in mutant genes (Coyle, 1996). These gains (or losses) of functional mutations appear to be relatively common in brain disorders such as Huntington's disease and spinobulbar muscular atrophy. The CAG repeats in the HD gene have been found in thousands of patients from ethnically diverse populations (Squitieri et al., 1994). Unstable mutations, due to trinucleotide repeats, are responsible for brain diseases characterized by increasing severity and earlier age of onset across generations. Expansions of CGG repeats are associated with the fragile X syndrome, the most common form of inherited mental retardation. Besides moderate to profound mental impairment, most males with fragile X syndrome show hyperactive behaviour, difficulties with concentration, and autism-like behavioural abnormalities (Jones et al., 1994). Spinal and bulbar muscular atrophies (SBMA) are a group of disorders characterized by loss of motor neurones in the spinal cord and lower cranial nerves. The clinical findings of gynaecomastia and impotence have led to the hypothesis that this disorder may result from a dysfunction of the androgen receptor which is expressed by spinal and cranial motor neurones (Mhatre et al., 1993). Expansion of the androgen receptor CAG repeat in SBMA families is the mutational mechanism responsible for the phenotype (Igarashi et al., 1992).
Anticipation, which was first reported for Huntington’s disease, has also been observed in psychiatric disorders such as affective disorders and schizophrenia. Whether or not unstable mutations are present in schizophrenia is today a subject of major debate. Further studies on extended populations are needed to confirm preliminary results that detected this type of mutation in small groups of schizophrenic and depressed patients (Ross, 1999). An expansion in a protein whose identity is yet unknown, has recently been found in a small group of patients with childhood onset schizophrenia (Morinieri, 1999). As the same reactive band has been seen in an individual with depression (Joyber et al., 1999), it is possible that schizophrenia and affective disorder may share some components of genetic variability. However this expanded band was not found in all families characterized by anticipated onset of schizophrenia, and in fact childhood onset schizophrenia is rare and sporadic. This could mean that, as the clinical anticipation of schizophrenic and affective symptoms, chiefly in bipolar disorder, has been described by many authors (Berrettini, 1997), other triplets repeat loci contribute to the etiology of schizophrenia and affective disorders.

Progress in molecular genetic techniques has led to an increasingly common interest that is shared by psychiatrists and neurologists in fundamental genetic mechanisms that can elucidate both the functioning of key aspects of the central nervous system and can shed light on the pathophysiology and pathogenesis of diseases of the CNS. Studies on the genetic basis of Huntington’s disease has led to the discovery of a new peptide, named “huntingtin”, that has a specific type of genetic defect, consisting of increasingly large numbers of nucleotide repeats; higher repeats lead to a defective form of the protein in Huntington’s disease. This type of molecular genetics strategy is now being applied to psychiatric disorders and has led to progress in the field of psychiatric genetics. Variations in the number of tandem repeats of the dopamine receptor subtype DRD4 have been associated with personality traits such as novelty seeking, drug dependence, and attention deficit hyperactivity in children (Benjamin et al., 1996; Ebstein et al., 1996; Kotler et al., 1997; LaHoste et al., 1996). Additionally, molecular genetics techniques such as genome screening have led to the elucidation of the genetics of some neurological disorders, including familial Parkinson’s disorders, that are now known to be due to a defect in the α-synuclein gene (Polymeropulos et al., 1997). Thus, progress in molecular genetics has led to an elimination of the traditional barriers between the approaches to and study of psychiatric and neurological disorders.
Modern genetic linkage studies allowing the identification of a gene effect based upon its close spatial relation with a defined inheritable marker have expanded the number of examples of the existence of relevant genes in neurological and psychiatric disorders. The finding of presenilin alterations on chromosome 1 and 14 in Alzheimer's disease is an example of such an approach.

Also, some genes may contribute to modifying disease vulnerability rather than cause the disease. In addition, life experience and environment, in particular during development, can strongly interface with the genetic background of an individual to produce positive or negative outcomes. An example of such an interface are genes related to sensitivity to substances of abuse, among which alcohol is the best studied.

Furthermore, the influence of environmental stimuli is so profound that it can induce changes in synaptic strength and also in the number of neurones, as shown by the observation that laboratory animals raised in a stimulus-rich environment have an increased number of synapses compared to control animals living in a standard cage.

**NEW EXPERIMENTAL APPROACHES AND EMERGING FIELDS**

Psychiatric disorders were originally classified as those that did not have a neuropathological basis. In the last two decades considerable progress in molecular neurobiology has led to the use of techniques such as *in situ* hybridization histochemistry to identify, at the cellular neuroanatomical level, areas of the brain that express specific genes. This has resulted in renewed interest on the part of neurobiologists in studying at the cellular level specific functional neuroanatomical pathways that may be dysregulated in psychiatric disorders. The same methods can be applied to animal brain and facilitate studies that bridge clinical and preclinical work. For example, the identification at the level of gene expression of a functional neuroanatomical circuitry of neurohormones, neuropeptides, and their receptors, that mediates the response to stress (Gold et al., 1996) has led to studies of those same circuits in postmortem brain specimens from subjects who had a lifetime diagnosis of major depressive disorder.

The possibilities of preclinical research are expanded nowadays because of the possibility of replicating in experimental animals genetic defects identified in human diseases. Studies in so-called transgenic animals involve rigorous examination of the effects of the gene concerned at cellular and systemic level, including effects on animal behaviour.

It has only recently been recognized that links between brain and periphery are much more extended than previously thought and
include the feedback signalling provided by several hormones produced by endocrine glands and organs, as well as the traditional neuroendocrine control exerted by the brain. The neuroendocrine network provides a moment-to-moment integration of signals from the environment and internal milieu. Correct information transfer at this level is important for normal brain functioning, and alterations or dysfunctions may affect "mental fitness" as does a direct CNS disturbance. A well-known example of such interactions are the changes of mood that are associated with the menstrual cycle. More recently the importance of neuro-immune interactions has also become evident.

Diseases affecting brain function that have traditionally been classified in distinct categories such as vascular (stroke), degenerative (Alzheimer's), infectious (neuroAIDS), inflammatory (multiple sclerosis), traumatic, psychiatric (affective disorders and schizophrenia) may all share common pathophysiological pathways that involve brain cytokines and growth factors (Cacabelos et al., 1994; Chiang et al., 1996; Huell et al., 1995; Licinio et al., 1993; Rothwell et al., 1996; Vikovic et al., 1994). For these reasons the field of neuro-immune interactions is currently one of the fastest-growing areas of neuroscience. For several decades interest in this topic was rather limited for several reasons. First, cytokines were molecules discovered and studied by immunologists who were mainly interested in their role in the regulation of peripheral immune responses. On the other hand, because cytokines were not molecules traditionally associated with brain function neuroscientists were not eager to shift their focus towards this newly emerging field. However, in the last five years, very rapid progress has been achieved in our understanding of the mechanisms through which peripheral immune mediators affect the functioning of the brain. The integration and differential regulation of central and peripheral cytokine compartments is a key element for the optimal functioning of the immune and nervous systems. It is relevant for the pathophysiology not only of diseases that have traditionally been conceptualized as disorders of the peripheral immune system, but also for brain disorders such as multiple sclerosis, stroke, brain trauma, neuroAIDS, Alzheimer's disease, and psychiatric disorders (Rothwell et al., 1996; Wong et al., 1996). The identification of neuroanatomical pathways and molecular mechanisms for the effects of cytokines in brain disorders has led to considerable progress in the understanding of the impact of inflammatory mediators in brain function, and has further eroded the traditional boundaries between psychiatry and neurology.
BRAIN IMAGING
Imaging of the brain has made it possible to understand structural and functional relationships between brain structure, cerebral blood flow, receptor binding and normal brain function and neurological and psychiatric disorders. Thus, techniques such as single photon emission computed tomography (SPECT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are revealing a functional neuroanatomical basis for neurological and psychiatric disorders. Interestingly, PET scanning has shown that treatment of psychiatric disorders by psychotherapy results in specific changes in brain metabolic activity (Scwhartz et al., 1996). A careful study of the treatment of obsessive-compulsive disorder with psychotherapy showed bilateral decreases in caudate glucose metabolic rates that were greater than those seen in poor responders to treatment. These results confirmed the presence of changes in caudate nucleus function with behaviour therapy for obsessive-compulsive disorder and support the current view that a prefrontal cortico-striato-thalamic brain system is implicated in the mediation of symptoms of obsessive-compulsive disorder. It has now become increasingly clear that psychotherapy affects specific brain circuits that are relevant to psychiatric disorders. Progress in these fields will facilitate a better understanding of psychiatric and neurological disorders at the molecular, cellular, structural, anatomical and functional levels. This will pave the way for considerable progress in the diagnosis, treatment and prevention of CNS disorders. It will also greatly reduce or eliminate the artificial boundaries between neurology and psychiatry that have resulted from an incomplete understanding of brain structure and function.

New developments in the understanding of brain metabolism have also contributed to bridging the gap between neurology and psychiatry. For example, imaging methods that map the metabolic activity of the brain are now widely used in the investigation of the biological basis of both psychiatric and neurological disorders. Thus, recent developments in PET and fMRI have made it possible to visualize brain areas that are activated by a variety of sensory, motor or cognitive tasks. This technological progress has allowed a kind of in vivo functional neuroanatomy which has led to the identification of neural circuits subserving specific brain functions. Metabolic processes linked to neuronal activity, such as blood flow, glucose utilization and oxygen consumption, provide the signals detected with most functional brain-imaging techniques. These metabolic indices have been examined in a variety of psychiatric and neurological disorders. For example, PET scanning has been used to diagnose brain tumours, a traditional neurological diagnosis, and has
also led to the concept of a prefrontal cortex defect in schizophrenia, a typical psychiatric disorder. The metabolic mechanisms that underlie the FDG-based PET imaging at the cellular and molecular levels have been intensively studied by Magistretti and colleagues (Magistretti and Pellerin, 1996). Their observations point to the critical role of a particular glial cell type, the astrocyte, in coupling neuronal activity to glucose utilization. Indeed it appears that in response to glutamate released by active neurones, glucose is predominantly taken up by specialized astrocytic processes, the end-feet, which surround brain capillaries; glucose is then metabolised to lactate, which provides a preferred energy substrate for neurones. Thus, glutamate stimulates glucose uptake and lactate production from astrocytes. This effect is mediated by glia-specific glutamate transporters, not by glutamate receptors, indicating a novel signalling mechanism for glutamate. As glutamate is released by active synapses, this mechanism provides a direct link between synaptic activity and energy metabolism. Those studies indicate that a tightly regulated neuron-glia interaction controlling energy metabolism appears to exist in the brain. These new data support the notion that astrocytes markedly contribute to the FDG-PET signal. This perspective may also provide renewed insights for interpreting the results of FDG-PET studies in neurological and psychiatric disorders.

CONCLUSIONS
In the last five decades much progress has been made in understanding the mechanisms underlying neurological disorders and also, to a lesser degree, psychiatric disorders. This growth of knowledge parallels the basic findings on the cellular membrane, the transport pathway and the basis of nerve excitation. Particularly important are the cellular membrane in the nervous system and the spatial and temporal presence of receptors and their involvement in the transduction of signals leading to physiological events. The activity of drugs at the level of the nervous system has been carefully studied with a view to improving their safety. The discovery of genetic disorders related to neurological and psychiatric disorders will certainly provide new tools for the control of some diseases.

Knowledge of genetic alterations makes it possible to generate transgenic mice or other animal models with the features of particular disorders. Further genetic studies may provide new perspectives for research that will enhance our understanding of psychiatric diseases, leading to more effective treatments and eventually to preventive strategies. In addition to their scientific and clinical relevance, studies using the tools of contemporary genetics that were so successful in the field
of neurology may finally lead to the full scientific and clinical integration of psychiatry and neurology. A limitation of current studies and a challenge to investigators is presented by the heterogeneity of psychiatric disorders and by the issue of genetic stratification. However, the progress achieved so far will be accelerated by new advances in the rapidly growing area of research on genes, behaviour, and health.

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GENETIC ADVANCES IN PSYCHIATRIC DISORDERS

Michael J. Owen and Alastair G. Cardno

INTRODUCTION
The genetics of mental illness has long been of interest and advances have been made in both quantitative and molecular genetics. In this chapter we give an overview of the methodological advances that have been made, and then describe the state of progress for specific examples of psychiatric disorders. We then discuss some of the implications of current and potential advances in the field.

QUANTITATIVE GENETICS
At the phenotypic level, studying the contribution of genetic and environmental factors to psychiatric disorders is based on information from family, twin and adoption studies (McGuffin et al, 1994). This has been facilitated by the adoption of a biometrical modelling approach (Jinks and Fulker, 1970), developed primarily in behavioural genetics. Twins are most often studied because of their genetic informativeness, although other relative groups can also provide valuable information. In behavioural genetics the variables studied are commonly quantitative, and modelling is typically by maximum likelihood methods, based on the variances and covariances of variables. In contrast, psychiatric disorders are generally defined categorically as the presence or absence of a diagnosis. However, categorical variables can be modelled by assuming a liability-threshold model (Falconer, 1965) in which multiple genetic and environmental factors form a continuous underlying liability, with individuals developing the disorder if their liability crosses a threshold. Liability and the position of thresholds are inferred from the risk of illness in relatives of affected individuals and the population risk for the disorder. The observed findings are compared with those expected under models specifying various patterns of genetic and environmental influences, and in this way specific hypotheses can be compared statistically. However, the power to differentiate between models is lower using categorical than quantitative variables (Neale et al, 1994).
The development of computer programs such as Lisrel (Jöreskog and Sörbom, 1989) and Mx (Neale, 1997) has greatly facilitated this type of analysis. In addition to univariate analyses, where a single disorder is studied, these programs can perform multivariate analyses to investigate the genetic and environmental relationship between disorders that show significant comorbidity (e.g., major depressive disorder and generalized anxiety disorder). Aspects of clinical variation within disorders can also be studied (e.g., age of onset), as may models involving, for example, repeated measures over time, and genotype-environment interactions. However, analyses which include such complexities may require very large sample sizes to differentiate between models.

These methods are aimed primarily at quantifying the genetic and environmental variation in liability to disorders. If a significant genetic contribution (heritability) is present, segregation analysis may be used to predict whether the genetic liability involves a single gene alone, multiple genes each of small effect, or a mixed model (Morton and MacLean, 1974). Again, there is more power to do this with quantitative than categorical definitions of disorders.

MOLECULAR GENETICS
Most progress has been made in this field for disorders where the genetic pathology is relatively easy to detect (e.g., the gross chromosomal abnormalities causing Down’s syndrome), or is a single gene disorder (e.g., Huntington’s disease and single gene causes of mental retardation). The latter includes Mendelian subforms of disease, as is found in familial Alzheimer’s disease and familial prion diseases. Progress in these diseases has also been helped by advances in knowledge about their pathophysiology, which has allowed the identification of strong candidate genes, such as amyloid precursor protein (APP) in Alzheimer’s disease and prion protein (PrP) in familial prion diseases.

However, for most common psychiatric disorders the pathophysiology is unclear and the quantitative genetics suggests complex inheritance involving multiple genes. For disorders such as schizophrenia, bipolar disorder, major depression and anxiety disorders no biological markers are yet available, and the diagnosis rests on the clinical syndrome. The development of operational diagnoses has allowed such disorders to be diagnosed reliably, and studies in genetic epidemiology have allowed estimates of the size of the genetic effect to be made and the genetic relationship between diagnoses to be classified. These advances have helped to define working phenotypes for molecular genetic studies, with a view to refinements when susceptibility genes are identified.
The potential for molecular genetic studies has been revolutionized by the rapid progress made in recombinant DNA technology. So far, advances in this field have been applied most to linkage and association studies of schizophrenia and bipolar disorder. Linkage studies are based on the principle that if a genetic marker and a disease gene lie close together on the same chromosome they will tend not to be separated by recombination during meiosis, and their alleles will be inherited together more often than the 50% of occasions predicted by Mendel’s law of independent assortment. Linkage studies of the functional psychoses initially adopted the parametric approach (Morton, 1955) that had been successful in the study of single gene disorders. Such studies are based on families with affected members in multiple generations (multiplex families), and seek evidence for selective inheritance of a marker allele in affected individuals. The best-fitting linkage model is usually calculated by the lod score method, where a lod score of greater than 3 is conventionally considered to be significant evidence for linkage, while a score below -2 excludes linkage. The parametric linkage approach assumes that a single gene of major effect is segregating in each family, and requires specification of genetic parameters, such as gene frequencies and penetrances, which are unknown for most psychiatric disorders. In view of this, and a lack of consistently replicated linkage results, the emphasis has now shifted to non-parametric approaches. Most commonly this involves the study of affected sib-pairs, where linkage is suggested by pairs inheriting the same alleles from their parents at a particular locus more often than expected by chance (Suarez et al, 1978; Holmans, 1993). Non-parametric linkage approaches make no assumptions about the mode of inheritance, and the study of affected relative pairs only has advantages in disorders such as schizophrenia where unaffected individuals can apparently carry a full complement of disease genes (Gottesman and Bertelsen, 1989).

The emphasis on non-parametric linkage has coincided with a move away from investigating linkage with specific candidate genes, to positional cloning approaches, where genes are identified by their location without prior knowledge of their function (Collins, 1992). Increasingly this is likely to be based on the results of systematic genome-wide linkage studies. The potential for such genome scans has been greatly enhanced by the identification of large numbers of highly polymorphic microsatellite markers, comprising simple repeat sequences of nucleotide bases (e.g. CA repeated a variable number of times). A genome scan for a functional psychotic illness may involve over 100 affected sib-pairs, typed for several hundred genetic markers spaced at 10-20 cM intervals throughout the genome.
Genome scans have also been facilitated by advances in statistical methods. In addition to single point analyses, where the information relating to each marker is considered separately, programs for multipoint analyses have been developed (e.g. MAPMAKER/SIBS: Kruglyak and Lander, 1995) that can give a distribution of lod scores, e.g. at 1 cM intervals, based on the combined information from all markers on a chromosome. Simulation studies allow the genome-wide significance of results to be estimated, which helps to account for the many statistical tests involved in a genome scan, although independent replication is still required for results to be treated with confidence (Lander and Kruglyak, 1995).

Association studies are the other main approach to locating genes involved in psychiatric disorders. Association and linkage studies can be seen as complementary, and are often carried out in tandem. While linkage studies are based on patterns of inheritance in families with multiply-affected members, association studies are population-based, and seek evidence that a marker allele is more common in affected than in unaffected individuals (Woolf, 1995). This approach has more power to detect genes of small effect than linkage, but the allelic marker must itself be contributing to the disease, or be in linkage disequilibrium with a disease gene, that is, so close that the two alleles are selectively inherited together in the general population. The distance over which allelic association can be detected varies with, e.g. the recombination frequency in the particular chromosomal region, and the length of time since the original mutation causing the allelic variation took place, but is typically about 1 cM (Owen et al, 1997). At the present time, therefore, association studies focus particularly on candidate genes. However, the lack of very strong candidates for the functional psychoses and neuroses, and the risk of statistical false positives, e.g. from multiple testing, mean that the prior odds against finding a true association are high. Therefore, as with linkage, replication of results is important (Owen et al, 1997).

In addition to statistical false positives, apparent allelic association may occur due to population stratification, e.g. where ethnic differences between illness and control groups result in differences of allele frequencies unrelated to the etiology of the illness. A well-matched control group is therefore essential. The risk of finding a spurious association due to population stratification can be reduced by using family-based association techniques, such as the haplotype relative risk approach (Falk and Rubinstein, 1987). Here, the frequencies of alleles transmitted to affected offspring by parents are compared with non-transmitted alleles. However, parents must be available for typing, and the sample should still be as ethnically homogeneous as possible (Owen et al, 1997).
In addition to a search for candidate genes, association studies are likely to be increasingly used to narrow down candidate regions identified through linkage, by searching for linkage disequilibrium with densely-packed markers, e.g. at 1 cM intervals, in the region of interest. The potential for this use of association studies has been enhanced by the identification of increasing numbers of single nucleotide polymorphisms (Wang et al, 1998), and the prospect that all genes and their common polymorphisms will be identified within the next two decades by the Human Genome Project. Again, the issues of multiple testing and replication are pertinent.

In both linkage and association studies establishing the significance of results is complex, and consistent replication of results is probably the best benchmark. However, failure to replicate a suggestive result may be due to the small effect size of individual loci, or to various forms of genetic heterogeneity, in addition to the occurrence of a statistical false positive (in some cases a consequence of multiple testing) (Owen et al, 1997). Therefore, it is important in such studies to calculate the statistical power and test for heterogeneity where possible.

Following the genetic mapping of a region of interest by linkage and association studies, a range of physical mapping techniques can be employed to identify the gene itself. For complex disorders this is not straightforward, and may require the investigation of larger genomic regions than has been necessary for single gene disorders (Owen and Craddock, 1996), but such work should be facilitated by the rapid progress being made in this area through the Human Genome Project.

SPECIFIC DISORDERS

Mental retardation

In most cases the causes of mental retardation are unknown (idiopathic), and are likely to involve heterogeneous mixtures of genetic and environmental factors (McGuffin et al, 1994). However, a number of specific genetic disorders are associated with mental retardation, including gross chromosomal abnormalities (e.g. Down’s syndrome), and single gene disorders involving autosomal dominant (e.g. tuberous sclerosis), recessive (e.g. phenylketonuria) or X-linked (e.g. fragile X syndrome) inheritance. Recently, considerable progress has been made in identifying the molecular basis of X-linked disorders.

Fragile X syndrome is the second most common known genetic cause of mental retardation after Down’s syndrome, with prevalences of 0.3-1 per 1000 males and 0.2-0.6 per 1000 females (Hirst et al, 1992). In addition to mental retardation the syndrome most character-
istically includes an elongated face, large everted ears and macro-orchidism (Hirst et al, 1992). The fragile site (FRAXA) was originally identified as a non-staining area on the long (q) arm of the X chromosome (Sutherland, 1997). The syndrome shows unusual patterns of inheritance. For example, the fragile site can be transmitted via an unaffected male to his carrier daughter (both of whom often do not have the fragile site themselves) to the daughter’s affected male offspring. The molecular basis of fragile X syndrome involves unstable expansion of a repeat sequence of the nucleotide bases, CGG, at the FRAXA site. In unaffected individuals the sequence is repeated up to about 50 times. Carrier females and normal transmitting males have longer repeat sequences, called premutations. When the gene is then inherited from a female carrier the sequence becomes longer still and the syndrome is expressed when the number of repeats exceeds about 230 (Pieretti et al, 1991). This causes inactivation of the FMR-1 gene, in which the expansion occurs.

Other families without expanded trinucleotide repeats at FRAXA have more distal fragile sites, FRAXE (Sutherland et al, 1992) and FRAXF (Hirst et al, 1993), which contain mutations involving CCG repeat expansions. Repeat expansions at the FRAXE site inactivate the gene, FMR-2, which is associated with non-specific mild mental retardation (as opposed to the syndromal mental retardation of FRAXA, which also includes physical features). Numerous other genes are thought to be involved in Z-linked non-specific mental retardation (Antonarakis and Van Aelst, 1998). Recently, two of these have been identified by positional cloning (BillUART et al, 1998; D’ADAMO et al, 1998), both of which encode proteins of the Ras superfamily of GTP-binding proteins, which have a wide range of biological functions (Antonarakis and Van Aelst, 1998).

At the phenotypic level, characteristics associated with genetic disorders may be studied with a view to identifying a behavioural phenotype (Flint and Yule, 1994). The best-established example is the self-mutilating behaviour of the autosomal dominant disorder, Lesch-Nyhan syndrome. However, finding a robust behavioural phenotype for fragile X syndrome is likely to be much more complex in view of the large sample size required for a definitive study (Flint, 1998).

**Dementias**

Studying the genetics of dementias is made relatively difficult by the late onset of disease in most cases. This means that potential probands may die before expressing the disease, while their relatives may not yet be ill, or be affected but dead at the time of study (McGuffin et al,
In spite of such difficulties, great progress has been made, particularly helped by the identification of single gene forms of dementia, and by the existence of strong candidate genes.

The genetic basis of Alzheimer's disease, the commonest form of severe dementia, is discussed in the chapter by Wolozin. Briefly, a number of families were observed where early-onset Alzheimer's disease apparently segregated as an autosomal dominant disorder. It was subsequently found that in some of these families the disease was related to mutations in the gene for the amyloid precursor protein (APP) (Goate et al, 1991), the substrate for beta-amyloid, which is an important constituent of the characteristic senile plaques found in large numbers in the brains of affected individuals. In other families, where such mutations were not identified, mutations of a novel gene named presenilin-1 (PS-1) were identified by positional cloning (Sherrington et al, 1995), and a search for homology with PS-1 resulted in the discovery of mutations in another gene (presenilin-1-2:PS-2) in other families (Rogaev et al, 1995). However, even taken together, mutations in these three genes account for only a very small fraction of Alzheimer's disease cases. A more general risk factor is possession of the e4 allele of apolipoprotein E (APOE), identified by allelic association studies (Strittmatter et al, 1993; Saunders et al, 1993). This allele has been estimated to account for around 17% of the variance in liability to Alzheimer's disease (Owen et al, 1994).

Prior to these advances in Alzheimer's disease, the genetic basis of Huntington's disease was established. This is caused by a single dominant gene with almost complete penetrance. An early positional cloning approach led to the discovery of linkage on chromosome 4p (Gusella et al, 1993). A number of genetic and physical mapping techniques, including allelic association, were then used to narrow the region of interest until the gene itself was identified (Huntington's Disease Collaborative Research Group, 1993). It was found to contain a CAG trinucleotide repeat, with the disease being expressed when the number of repeats is greater than 37. The number of repeats is negatively correlated with age of onset (e.g. Snell et al, 1993), and thus provides a molecular explanation for the phenomenon of anticipation, where a disorder has an earlier age of onset and/or severity in successive generations. The protein product of the gene has been identified (and called huntingtin), and its function is currently being investigated (Jones, 1996).

Genetic advances have also been made in the spongiform encephalopathies, which in humans include Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler syndrome (GSS). These are caused by a con-
formational change in a normal brain protein (PrP) (Fleminger and Curtis, 1997). CJD is usually sporadic, the conformational change occurring spontaneously, or transmission of CNS material, but familial forms also occur, while GSS shows an apparently autosomal dominant inheritance. Mutations in the gene encoding PrP have been found to segregate with the disease in some families with CJD and GSS (Owen et al, 1989; Hsiao et al, 1989):

Functional psychoses

An important genetic contribution to the etiology of schizophrenia has been shown by family, twin and adoption studies (Gottesman, 1991). Earlier studies, based on clinical diagnoses, have been confirmed using more precise operational criteria. It has also been shown that the inheritance is complex, with multiple genes probably being involved (O'Rourke et al, 1982; McGue et al, 1985). The clinical presentation of schizophrenia is very variable, but it is unlikely that genetically distinct subtypes exist within the disorder (McGuffin et al, 1987). However, schizophrenia shares familial, and probably genetic, liability with a range of other psychotic illnesses (Kendler et al, 1993a) and personality disorders, such as schizotypal personality disorder (Kendler et al, 1993b), collectively known as schizophrenia spectrum disorders. In view of this, narrow and broad approaches to phenotype definition are often taken. The resultant multiple statistical testing increases the risk of false-positive results, and highlights the importance of replication of results.

Parametric linkage studies in multiplex families have followed a disappointing pattern of suggestive findings followed by failure to replicate. An example is the investigation of linkage on chromosome 5q, following the report of an uncle and nephew who both had schizophrenia and shared a partial trisomy in this region (Bassett et al, 1988). An initial study found evidence for linkage (Sherrington et al, 1988), but others failed to replicate the finding (e.g. Kennedy et al, 1988; Aschauer et al, 1990; McGuffin et al, 1990), which has been thought in retrospect to be a statistical false positive rather than due to genetic heterogeneity (McGuffin et al, 1990). As more linkage studies are completed it seems increasingly likely that genes of large effect in schizophrenia are at best rare, and may be non-existent (McGuffin and Owen, 1996). A number of genome scans using non-parametric approaches are currently being performed (e.g. Straub et al, 1995; Williams et al, 1997; Wildenauer et al, 1997; Levinson et al, 1998), in some cases along with parametric analyses. Recent results from some genome scans and other studies provide suggestive evidence for linkage on chromosomes 6p (Straub et al, 1995), 8p (Pulver et al, 1995), and 22q (Pulver et al,
1994). However, despite collaborative efforts to replicate these results in comparatively large samples (Schizophrenia Linkage Collaborative Group for Chromosomes 3,6 and 8, 1996; Schizophrenia Collaborative Linkage Group, 1996), in no case has confirmation achieved a degree of statistical certainty sufficient to warrant physical mapping studies.

Many association studies have focused on genes coding for neurotransmitters implicated in the therapeutic effects of antipsychotic drugs. Although most studies have been negative, there is evidence of an association between schizophrenia and homozygosity for a polymorphism in the dopamine DRD3 receptor gene (Crocq et al. 1992; Mant et al, 1994). A recent meta-analysis of relevant studies showed a significant, but small effect (odds ratio = 1.21) (Williams et al, 1998). Another study, involving the collaboration of seven European centres, found an association with a polymorphism of the 5HT2A receptor gene (Williams et al, 1996), again of small effect size (odds ratio = 1.7). This finding has also been confirmed in a meta-analysis (Williams et al, 1997), and in a TDT family-based association study (Spurlock et al, 1998). The functional significance of both the DRD3 and 5HT2A receptor gene polymorphisms is currently being investigated.

A possible clue to the type of molecular genetic mechanism involved in schizophrenia has come from the study of trinucleotide repeat sequences of the kind implicated in Huntington’s disease and other disorders with neurological involvement. The presence of anticipation in schizophrenia is difficult to confirm because of potential ascertainment biases (Asherson et al, 1994), but several studies suggest that it may be present (e.g. Bassett and Honer, 1994). At the molecular level, the RED technique (Shalling et al, 1993), which measures the longest CAG/CTG repeat in an individual’s genome, has been used to show that such repeats tend to be longer in people with schizophrenia than in controls (O’Donovan et al, 1995, 1996; Morris et al, 1995). However, the mechanism may differ from that found in the single gene disorders as repeat length appears unrelated to age of illness onset (Cardno et al, 1996). Specific genes where longer repeats are associated with schizophrenia are now being sought.

Progress in knowledge about the genetic basis of bipolar disorder has followed a similar course to that for schizophrenia (Kirov and Owen, 1998). Family, twin and adoption studies again indicate an important genetic component (Tsuang and Faraone, 1990), and the inheritance appears complex and to involve multiple genes (Craddock et al, 1995). An important issue regarding the phenotype is whether or in what way bipolar disorder (BP) and unipolar depression (UP) are genetically related. Relatives of probands with BP tend to have an elevated risk of BP
and UP, while relatives of probands with UP tend only to have an elevated risk of UP (McGuffin and Katz, 1986). However, BP may not simply be a more severe form of disorder on the same liability continuum as UP (Tsuang and Faraone, 1990).

Early findings of linkage to "classical" markers on the X-chromosome, such as colour blindness, glucose-6-phosphate dehydrogenase deficiency, and the gene for coagulation factor number IX, have not been consistently replicated using more recent molecular approaches. However, one study has shown suggestive evidence of linkage on the X-chromosome in a large family from a genetically isolated part of Finland (Pekkarinen et al, 1994). Early findings from studies of an extended Amish family in Pennsylvania suggested linkage on chromosome 11q (Egelund et al, 1987) but, in this instance, the results lost significance when additional family members became ill. A subsequent genome scan has revealed no definite areas of linkage in this family (Ginz et al, 1996), probably because the assumption that bipolar disorder in the family is due to a mutation in a single gene is incorrect. A number of other genome scans are in progress using parametric and/or non-parametric approaches. One completed scan found relatively strong evidence for linkage on chromosome 4p in one large multiplex family (Blackwood et al, 1996). Other recent suggestive findings involve chromosomes 12q (Craddock et al, 1994), 18 (Berrettini et al, 1994), and 21q (Straub et al, 1994) but again none of these results is fully confirmed.

Association studies of candidate genes have shown evidence for an association between bipolar disorder and the serotonin transporter gene (Collier et al, 1996; Rees et al, 1997). As with schizophrenia, there is also some evidence that bipolar disorder shows anticipation (McInnis et al, 1993), and that CAG/CTG repeats are longer in affected individuals than controls (Lindblad et al, 1995; O'Donovan et al, 1995, 1996). This raises the intriguing question of whether schizophrenia and bipolar disorder share one or more susceptibility genes in common.

Neuroses
A genetic contribution has been established for most disorders in this area including major depressive disorder, anxiety disorders, obsessive compulsive disorder and anorexia nervosa, although generally with lower estimates of heritability than for the functional psychoses (McGuffin et al, 1994). The commonest presentation of psychiatric morbidity involves a mixture of depression and anxiety symptoms. In order to investigate this comorbidity, Kendler et al (1992) applied bivariate model fitting to data on twins with major depressive disorder and generalized
anxiety disorder. This suggested that the genetic liability to both disorders is the same, while individual-specific environmental factors are only partly shared, and common familial environmental factors play no role in the etiology of either disorder.

The complex relationship between genetic and environmental factors is illustrated by research into life events and depressive illness. It is well established that individuals tend to have an excess of stressful life events prior to a depressive episode (e.g. Brown and Harris, 1978). Life events tend to be thought of as purely environmental, but it has been found that the reporting of stressful life events by individuals with depression may be influenced by familial (McGuffin et al, 1988) and possibly even genetic factors (Kendler et al, 1993c; Thapar and McGuffin, 1996). This could be because someone prone to depression may perceive events as threatening, or behave in a way that exposes them to more adversity (Owen and McGuffin, 1997).

**Childhood psychiatric disorders**

This field has made good use of developments in quantitative genetics, often employing population-based twin registers and including quantitative approaches to the definition of phenotypes. A recent twin study from Virginia investigated attention deficit hyperactivity disorder (ADHD), conduct disorder, oppositional defiant disorder, overanxious disorder, separation anxiety, and depressive disorder in 1412 twin pairs (Eaves et al, 1997). The analysis included comparison of ratings by the twins, their parents, and their teachers, as well as investigation of sex effects and sibling interactions. Most measures showed small to moderate genetic effects, although ADHD appeared highly heritable. The largest sex difference was for separation anxiety, which showed a large genetic effect for girls but none for boys. The largest sibling contrast effects were for ADHD, which was thought to be the cause of the low dizygotic twin correlations that were found.

These results were generally consistent with other twin studies of psychiatric symptoms in childhood, such as anxiety (Thapar and McGuffin, 1994) and ADHD (Gjone et al, 1996). The study of depressive symptoms (Thapar and McGuffin, 1994) also showed an increase in genetic effects as children got older, emphasizing that heritability need not be static over time. Association studies of ADHD have shown positive results for polymorphisms of the dopamine transporter gene (Cook et al, 1995) and dopamine D4 receptor gene (LaHoste et al, 1996).

Autism is another disorder which has been shown to have an important genetic contribution to its etiology (Bailey et al, 1996). A re-
cent non-parametric genome scan (International Molecular Genetic Study of Autism Consortium, 1998) using affected sib-pairs and other affected relative pairs, found a maximum multipoint lod score of 3.55 or chromosome 7q. Replication of this result is awaited.

**Alcohol dependence**

Family, twin and adoption studies have suggested an important genetic contribution to this disorder (McGuffin et al, 1994). On the basis of this, a collaborative group (COGA) has performed a genome-wide non-parametric linkage study of sib-pairs (Reich et al, 1998). In addition to the affected sib-pair analysis, a regression approach was adopted (Haseman and Elston, 1972) that included unaffected sibs, a variant of which has been successful in detecting linkage for quantitative measures of reading disability (Cardon et al, 1994). The most suggestive loci were found on chromosomes 1 and 7, with maximum multipoint lod scores of 2.9 and 3.5, respectively. Replication of these results is awaited.

**IMPLICATIONS OF FINDINGS**

**Genetic counselling**

Genetic counselling is ‘the process by which patients or relatives at risk of a disorder that may be hereditary are advised of the consequences of this disorder, the probability of developing or transmitting it, and of the ways in which this may be prevented or ameliorated’ (Harper, 1993). The implications of advances in psychiatric genetics are likely to differ according to the disorder in question, particularly whether it is caused by a single gene or has a more complex multifactorial etiology.

Many genetic counselling implications for single gene disorders have been tackled in the case of Huntington’s disease (Harper et al, 1996a). Since the characterization of the responsible gene, direct mutation testing of individuals has allowed diagnosis and prediction of future disease to be made very accurately. Prenatal exclusion testing can also be performed. Pre-symptomatic testing is carried out in the context of pre- and post-test counselling. Prior to testing care is taken to ensure that the individual has been referred appropriately, in particular that the individual is fully informed about, and consenting to, the test. There is an international consensus not to test children below the age at which they themselves can give informed consent (World Federation of Neurology Research Committee. Research Group on Huntington’s Disease, 1989). The implications for other family members are also explored, especially where the risk status of, e.g. the tested individual’s
parents, may be affected by the information from the test. In practice, only 9-15% of those who have expressed an interest in pre-symptomatic testing in England and Wales have actually proceeded to be tested (Harper et al, 1996b). This may partly be because there is currently no effective treatment for Huntington’s disease.

The implications for other single gene disorders are likely to be similar to those for Huntington’s disease. Direct mutation testing is available for fragile X syndrome (Hirst et al, 1992), and could be developed for other diseases, such as the cases of familial Alzheimer’s disease caused by a single gene mutation. However, for the majority of cases of Alzheimer’s disease the etiology is more complex, making prediction of risk less accurate. APOE is the best-established allelic risk factor for Alzheimer's disease in the general population, but it is neither necessary nor sufficient for the development of the disease, and APOE genotyping for diagnostic or predictive testing has not been recommended by a recent working group (Scourfield and McGuffin, 1998).

The genetic basis of most other common psychiatric disorders is also complex. Risk estimates are based on epidemiological risks to relatives of affected individuals, and it should be borne in mind that heritability is concerned with population variation in disease liability and does not have a simple meaning at an individual level. Most susceptibility loci are likely to be of modest effect size, and so only have a small effect on an individual’s risk. For example, the risk of schizophrenia for an individual with an affected sibling is around 10%. Assuming the allelic association between schizophrenia and 5HT2A turns out to be true, having the susceptibility allele only increases the individual’s risk to 12.3 (Scourfield and McGuffin, 1998). Furthermore, even monozygotic twins have concordance rates for schizophrenia of less than 50%, so even knowing the allelic status of an individual for all relevant susceptibility genes is unlikely to allow prediction of risk comparable with what is possible for single gene disorders. For most psychiatric disorders, environmental factors appear to be important in addition to genetic liability. Therefore, persons with a strong family history of alcohol dependence may wish to be cautious about their level of alcohol consumption, but it is important that such decisions, and others concerning e.g. marriage and having children, are made by the persons themselves - the counsellor’s role is to provide the relevant information to help them make their own decisions.

**Dangers and misconceptions**

These have been discussed recently by a number of authors (e.g. McGuffin et al, 1994; Farmer and Owen, 1996; Owen and McGuffin,
The discovery of a susceptibility gene for a complex disorder may be misinterpreted as its being the sole cause of that disorder, that its presence inevitably results in disease, and that its consequences are invariably ‘bad’. In fact, as discussed above, most susceptibility genes for psychiatric disorders are likely to have only small effects in themselves, may be common in the general population, and may have biologically useful functions. Even genes of major effect may have beneficial properties, the best-known example being the gene for sickle-cell disease, which gives some protection against malaria.

Such misconceptions may give rise to fears that non-biological treatments will be neglected. However, the effectiveness of biological versus non-biological treatments does not follow in any simple way from the etiology of a disorder. It is well established that disorders with an important genetic contribution to their etiology can have effective non-biological treatments, e.g. dietary treatment for phenylketonuria and cognitive therapy for depressive illness.

More serious is the possibility, based on all too recent history, of calls to rid the population of disease-causing genes by means of eugenic programmes. Counter-arguments include the fact that we are all likely to carry susceptibility genes for a variety of diseases, but may not become ill, e.g. because we do not have enough of the relevant genes or have not come into contact with relevant environmental factors. The complex effects of disease susceptibility genes, including potentially beneficial ones, and possibilities of advances in treatments also argue against the value of such programmes. However, fundamentally these are moral and political issues. The role of the psychiatric geneticist is primarily to provide the necessary public information to allow informed debate. This also applies to the use of information generated by research, including disclosure, e.g. to insurance companies and employers, and to the practice of psychiatric genetic research itself. Probably the best safeguard against abuse of genetics is for researchers to be open about their current work and future intentions (Harper et al., 1996a). The regulation of this work, as society sees appropriate, may then be based on the fullest possible knowledge.

**Advances in diagnosis and treatment**

It is unlikely that some form of gene therapy will be useful for most psychiatric disorders (Rutter and Plomin, 1997). It may have a limited role in the treatment of some single gene disorders, but not for complex disorders where the susceptibility genes have small individual effects and potentially a range of biological functions. The main benefits of characterizing the genetic contribution to most psychiatric disorders
are likely to derive from a better understanding of the pathophysiology of these disorders, including the relationships between biological and environmental factors.

It is hoped that diagnoses based on such information will be more useful than at present for the prediction of effective treatment and prognosis, and that improved diagnosis will be aided by the discovery of biological markers for some disorders. Individuals at relatively high risk of developing a disorder may be identified and monitored regularly so that if they start to become ill they can be treated early. It is hoped that a better understanding of the pathophysiology of disorders will lead to more specific biological treatments, and a better understanding of environmental risk factors. In addition to better treatment once a disorder has developed, it is hoped that people who wish to reduce their risk of developing a disorder will have a greater choice of actions that they can take, e.g. prophylactic medication or lifestyle changes, in order to do so.

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MOLECULAR NEUROENDOCRINOLOGY AND ITS IMPACT ON BEHAVIOUR

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ABSTRACT
Genetic factors do not account for all the individual variability in the responses to internal or external stimuli. It is now accepted that environmental, psychosocial, and behavioural factors all contribute to the biology of mental illness. Acute stress, defined as a “fight or flight” situation, and chronic stress, defined as the cumulative load of minor stresses, have long-term consequences. The autonomic nervous system, the endocrine system, particularly the hypothalamic-pituitary-adrenal (HPA) axis, as well as the cardiovascular and immune systems protect the organism from internal and external stressors. Responses to stress may result in hyperactivation or hypoactivation of all these complex systems. As the final product of the HPA axis, steroid hormones of the adrenal cortex regulate homeostasis and have effects in the brain by acting at the level of central nervous system (CNS) glucocorticoid receptors, modifying neurochemical and structural features of the brain. This review discusses the pivotal role of HPA axis hormones in the responses to internal and external stressful events.

HISTORICAL BACKGROUND
The existence of adrenal glands has been known for centuries, but both their functions and involvement in disease remained a mystery. It was only in 1855 that Thomas Addison (Addison 1855) first described a “dark skin disease”, a dermatological condition that he found connected with pathological modification of the adrenal glands. That disease had a fatal course, which was characterized by anaemia, general weakness and fatigue, disturbances in the digestive apparatus, enfeebled heart activity and a peculiar dark pigmentation of the skin. Addison’s great contribution was to show that this morbid picture made its appearance in persons the greater part of whose adrenals was destroyed. Addison’s work constitutes one of the basic contributions to our knowledge of endocrinology in general and to the role played by the adrenals in health
and disease. One year later, Brown-Séquard confirmed Addison’s observations, thus demonstrating the importance of these “small capsules”. Since then, a large amount of literature has recognized the importance of the adrenal cortex in glucose metabolism, through the secretion of specific hormones, called corticosteroids (Munck et al 1984).

Almost a century after Addison’s observations Hench et al (Hench 1949) discovered the therapeutic effects of corticosteroids on inflammatory diseases such as rheumatoid arthritis and asthma. The worldwide enthusiasm engendered by this achievement was reflected by the award in 1950 of the Nobel Prize in Physiology or Medicine to Edward Calvin Kendall (United States), Tadeus Reichstein (Switzerland) and Philip Showalter Hench (United States) “for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects”. That enthusiasm was later tempered by the observation that the therapeutic use of corticosteroids was accompanied by several side effects such as emotional lability, distractibility, anxiety, insomnia, depression, and cognitive impairment (Clark et al 1952). The hypothesis that steroid hormones might access the brain was first suggested in 1943 by Harris (1972). In 1968 McEwen et al reported that the hypophysiotropic area is not the only target for steroid action, but adrenal steroid receptors are also present in extrahypothalamic limbic regions in the rat brain. Finally in 1981 Vale and colleagues synthesized the corticotropin releasing factor (CRH), a 41 aminoacid neuropeptide since then known as corticotropin releasing hormone (CRH), in hypothalamic and extrahypothalamic sites (Vale et al 1981). From then on, a connection between steroid hormone secretion and psychopathology became clearer and clearer.

**HPATHALAMIC-PITUITARY-ADRENAL (HPA) AXIS**

The HPA axis involves a network of central and peripheral components, which includes corticotropin releasing hormone (CRH), urocortin (a CRH-related peptide), ACTH, and cortisol (corticosterone in rodents). The action of corticotropin releasing hormone (CRH) in the brain and in the periphery is mediated through multiple binding sites. There are five CRH receptor (CRHR) isoforms encoded by two distinct genes, CRHR	extsubscript{1a}, CRHR	extsubscript{1b}, CRHR	extsubscript{2a}, CRHR	extsubscript{2b}, and CRHR. Using *in situ* hybridization, our group conducted in 1994 a detailed study on CRH receptor type 1 in rat brain (Wong et al 1994). A strong autoradiographic signal was found over the pituitary, the granule cell layer of the dentate gyrus, the pyramidal cell area of the hippocampus, piriform cortex and cerebral cortex area. CRH receptor activation increases the intracellular levels of calcium and cAMP; however, the DNA responsive elements
CRE (cAMP responsive element) and CARE (calcium responsive element), both variants of the basic palindromic motif TGACGTCA, are not present in the promoter region of the proopiomelanocortin (POMC) gene, a major target of CRH bioactivity. This fact has led to the currently ongoing search for novel CRH-related transcription factors and their responsive elements in the POMC promoter.

The actions of CRH are modulated by a CRH-binding protein (CRH-BP), which serves as a decoy for bioactive CRH. A novel therapeutic strategy has involved using ligands that block the CRH-BP, which increases CRH bioactivity by decreasing the amount of CRH that binds to CRH-BP, thereby increasing the amount of CRH that is available to bind to signal-transducing receptors. The use of such CRH-BP ligands in an animal model resulted in improvement in learning and memory without stress-like side effects (Behan et al 1995).

The most recently discovered CRH receptor ligand in mammals is urocrin, a CRH-related peptide with close sequence homology to fish urotensin (Vaughan et al 1995; Wong et al 1996). It elicits a robust ACTH response after peripheral administration, and it interacts with CRHR1, CRHR2, CRHR3, CRHR4, and CRHR2 receptors and with CRH-BP (Behan et al 1996) and affects HPA function.

Brain CRH is elevated in several disease states including major depression, anorexia nervosa, and stroke; therefore, CRH receptor antagonists could be useful for the treatment of these diseases.

Central administration of CRH to the rat causes a series of coordinated events that can be considered adaptive to stress. These include activation not only of the HPA axis but also of autonomic activity, which results in increased blood glucose levels and higher heart rate and blood pressure. As a consequence, CRH administration facilitates symptoms of hyperarousal, such as avoidance and anxiety, while inhibiting feeding, sexual activity and sleep (Gold et al 1988a; Gold et al 1996). CRH secreted into the hypophysal portal circulation binds to CRHR1 eliciting the production of transcription factors which bind to CRH-responsive elements of the proopiomelanocortin (POMC) promoter, thus increasing POMC gene transcription. POMC mRNA is translated into many peptides, including ACTH and b-endorphin. ACTH secretion is also under the control of arginine-vasopressin (AVP); AVP is released from two sites in the hypothalamus: the parvicellular division of the paraventricular nucleus (PVN), where CRH is also formed; and the magnocellular neurons of the supraoptic nucleus (SON). Two major subtypes of AVP receptors have been identified (Jard et al 1987). V1-a receptors are widely distributed in the periphery and have been found also in the central nervous system (Dubois-Dauphin et al 1990).
V2 receptors are located predominantly in the renal collecting system, although evidence exists of their presence in the brain (de Wied et al 1991). Of importance has been the cloning of the V1-b receptor gene (Jard et al 1986), at which AVP is active in the anterior pituitary. Paradigms associated with HPA hyperresponsiveness, and ACTH release due to a novel stimulus in a chronic stress situation have been found to show a shift in the CRH/AVP ratio (Scott and Dinan 1998). AVP may be differently sensitive to glucocorticoid feedback restraint, as it shows a greater responsivity to chronic stressors.

Corticosteroid hormones act via intracellular receptors. The binding of the corticosteroid hormone to its receptor leads to a conformational change in the receptor and its dissociation from its binding protein (Beaulieu 1987). Translocation signals induce a dimerization of the receptor complex. This dimer enters the nucleus and binds to DNA, promoting transcription. Subsequently, changes in transcription lead to changes in translation of mRNA to protein, resulting in steroid-induced effects at the cellular level. Both type I and type II receptors have been cloned: type I has a high affinity for aldosterone, whereas type II has a high affinity for dexamethasone, a synthetic corticosteroid (Lupien and McEwen 1997), while it shows a lower affinity for aldosterone. In the brain, type I receptors are more widely expressed than type II, chiefly in the limbic system and in the brainstem motor nuclei. Type II receptors are present in the paraventricular nucleus and other hypothalamic nuclei, as well brainstem monoaminergic nuclei (Reul and de Kloet 1985). Both type I and type II are differently regulated by endogenous or exogenous steroids. Corticosterone down-regulates type II receptors, but up-regulates type I receptors (Swanson and Simmons 1989). Mineralocorticoids down-regulate both types, while spironolactone, a type I receptor antagonist, has the opposite effect (Luttge et al 1989). These effects on binding are accompanied by changes in mRNA levels, which could suggest a role for steroids in the turnover rate of the receptors (Reul and de Kloet 1985). Glucocorticoid action has been extensively studied in the hippocampus because it has an important role in memory and contains both types of receptors. The physiological responses of hippocampal neurons to corticosteroid administration range from modulation of excitability to regulation of neurogenesis and programmed cell death in the dentate gyrus, and atrophy of the Ammon’s horn (Lupien and McEwen 1997). High levels of type II receptor activation increases N-methyl-D-aspartate (NMDA) receptor expression in the hippocampus (McEwen 1996). NMDA regulates a variety of signalling pathways, ranging from localized, acute effects on receptor and calcium channel activities to long-term effects on gene transcription.
Adrenal steroids thus have distinct effects on hippocampal structure and plasticity that are important in learning, memory and cognition at different levels.

**HPA AXIS AND ENVIRONMENT AND STRESS**

Two important and contrasting types of relations exist in mammalian social systems, dominance relationships and social bondings. In a stable social system, characterized by established dominant individuals, a lower position in the hierarchy does not necessarily lead to an enhanced stress response. However, under conditions of unpredictability, where a stable hierarchy is absent, a condition of competition enhances a distinct response of the HPA axis and sympathetic-adrenal-medullary (SAM) axis, also known as the locus coeruleus/norepinephrine (LC/NE) system, the former through the excretion of proopiomelanocortin (POMC)-derived peptides, such as adrenocorticotropic hormone (ACTH), and cortisol, the latter through the production of norepinephrine. For optimal adaptation, the stress response should be acute or of a limited duration. Chronicity and excess of stress, on the contrary, lead to the “general adaptation syndrome” described by Selye in various experimental animals (Selye 1936). He later hypothesized that severe diseases of any kind could show symptoms of anorexia, loss of weight, depression of mood, hypogonadism, peptic ulcers, and immunosuppression. Detection of increased production of corticotropin releasing hormone (CRH) in severely ill subjects could contribute to explaining the pathogenesis of the “general adaptation syndrome”, as CRH can be responsible for every one of its symptoms.

Major depressive disorder, particularly recurrent depression with melancholic features, represents a model of dysregulation of the generalized stress response and shares many biochemical similarities with acute stress (Gold et al 1988a). Both conditions are associated with hyperactive HPA and SAM axes, resulting in elevated levels of hypothalamic CRH, enhanced cortisol and catecholamine secretion, increase of the catecholamine-induced interleukin-6 (IL-6) concentration (Papanicolaou et al 1998), inhibition of growth, thyroid, and gonadal systems, and suppression of the immune system.

Environmental factors can be considered stressful events that have been identified since the time of Hippocrates in the pathogenesis or exacerbation of many diseases, ranging from cardiovascular diseases to classical psychosomatic disorders and psychiatric disorders, such as major depression. Recent data from genetics, pharmacological, neurochemical and neuroanatomical studies provide an emerging and increasingly clearer picture of the contribution of biological and environmental
factors in the genesis of psychiatric disorders (Nemeroff 1996). Animal models have been developed to evaluate the role of social separation on behaviour and psychological responses. Since the mid-1970s (Suomi et al. 1975) many studies have shown that early separation of Rhesus monkeys from their peers causes a persistent despair reaction, that may represent an equivalent of depression in humans. Also in rats (Weinstock et al. 1978) social isolation can lead to enduring hyperactivity, aggressiveness (Valzelli and Bernasconi 1976), and impairment of performance (Jones et al. 1991). Female rats showed higher plasma corticosterone concentrations and lower anterior pituitary CRH receptor density in comparison with male rats. Male rats have demonstrated greater changes in response to social isolation than females. Thus, sex appears to represent an important variable in determining the behavioural response to social isolation. Unfortunately, research in humans to date has failed to provide a clear paradigm of response to early parental loss or social isolation as well a clear gender distinction. Moreover, research in humans has very frequently been biased by varying definitions for the precipitating events under study and methodological inconsistencies.

The development of responses to stress in the rat is undoubtedly influenced by the early postnatal environment (Meaney et al. 1996). Handling during the first week of life is a very simple paradigm: handled pups are simply removed from the nest for 3-15 minutes and then reunited with the mother; this does not represent a period of maternal deprivation, because, in nature, mothers are routinely off their nest for periods of 20-25 minutes during the day (Leon et al. 1978). Nonetheless this test is able to decrease behavioural fearfulness and HPA responses under conditions of stress. These effects persist through the whole animal life and may be a basis for stress-related diseases (Seckl and Meaney 1993). In a very recent study (Caldji et al. 1998) pups were unmanipulated and only maternal behaviour was observed. As adults, offsprings of high licking/grooming mothers showed: (1) significantly increased central benzodiazepin receptors in the central, lateral, and basolateral nuclei of the amygdala as well as in the locus coeruleus; (2) increased α₂ adrenoreceptor density in the locus coeruleus; (3) decreased CRH receptor density in the locus coeruleus. Altogether these findings suggest that maternal care during infancy is necessary to programme behavioural responses to stress in the offspring by modifying the plasticity of the neural system that mediates fearfulness. Spontaneously, one question arises: Is this true for humans too? In the last few years an accumulation of data has revealed a pre-eminent role of early life unfavourable experiences (Nemeroff 1999). In his work Freud highlighted (Roth 1998) the importance of early trauma on the later development of mood depression.
Freud himself stated in a radio recording for the British Broadcasting Corporation in 1939, shortly before his death: "People did not believe in my facts, and thought my theories unsavoury. Resistance was strong and unrelenting. In the end, I succeeded in acquiring pupils and building up an International Psychoanalytic Society. But the struggle is not yet over". Indeed, the struggle to integrate biological, genetic, and environmental factors in our understanding of the biology of mental illness is not yet over (Licinio 1998a).

Very recently Agid et al (1999) have shown that loss of a parent before the age of 17 years significantly increases the likelihood of developing major depression during adult life. More strikingly, the effect of loss due to separation was even worse than loss due to death, as was loss before the age of 9 years compared to loss experienced during adolescence. A significantly increased effect of early parental loss was also observed in patients with schizophrenia, again for loss before the age of 9 years. Enduring changes in behaviour have long been suggested by animal studies (Hinde and Spencer-Booth 1971). These are characterized by increased CRH concentrations, reduced CRH receptor concentrations in the pituitary, and increased levels of CRH mRNA in the hypothalamus (Coplan et al 1996). Moreover, glucocorticoid and mineralocorticoid receptors have been found to be down-regulated in adult rats which suffered from maternal deprivation during infancy (van Oers et al 1998). It is well known that CRH, through induction of somatostatin, inhibits growth hormone secretion. In fact, maternal deprivation damped growth hormone secretion in 2-day-old rats (Kacsóh et al 1997). In non-human primates certain rearing conditions in early life have been associated with increased cerebrospinal CRH levels. To our knowledge, no similar studies have ever been conducted on humans. It is conceivable that early parental loss may cause analogous alterations and enhance vulnerability to psychiatric illness.

**HPA AXIS AND VULNERABILITY TO ILLNESS**

Two distinct CRH systems coexist in the brain: one that is bound to glucocorticoids, and one which is not. The latter includes the central nucleus of the amygdala and the lateral bed nucleus of the stria terminalis, in which regulation of CRH gene expression is not linked to the paraventricular nucleus of the hypothalamus. This system is linked to clinical syndromes whose major symptoms are fear and anxiety (melancholic depression, post-traumatic stress disorder and abuse of psychoactive drugs). In the central nervous system, studies of stress hormone action in neurons of the hippocampus have led to new insights into the neurobiology of learning and memory by revealing the vulnerability of
neurons and glia cells to damage as well as the natural structural plasticity of hippocampal neurons, including dendritic remodelling and dentate gyrus neurogenesis (McEwen 1998). The concept of “allostatic load”, which means cost of adaptation, provides a new mechanistic framework for understanding the role of stress and other environmental mediators in health and disease of the brain, the immune system, and the metabolic and cardiovascular systems.

Although it has been known for some time that the human brain shows signs of atrophy in response to elevated cortisol and traumatic stress (Sapolsky et al 1986), only very recently brain imaging techniques have allowed us to see which structures are most affected. The human hippocampus is particularly sensitive in this respect, and shows greater changes than other brain areas, particularly in Cushing’s syndrome, recurrent depression, schizophrenia, post-traumatic stress disorder as well as in normal aging. It has been hypothesized that hippocampal atrophy in Cushing’s disease is reversible, and agents such as tianeptine and phenytoin can reverse hippocampal atrophy in conditions such as recurrent depression and post-traumatic stress disorder (McEwen 1997; Sheline, 1996; Sheline et al 1996). A modulatory role for glucocorticoids is also evident in the amygdala. In fact, the amygdala is involved in the emotionally influenced processes of learning and memory. Both type I (mineralocorticoid) and type II (glucocorticoid) adrenal steroid receptors are present in the central and median nuclei of the amygdala. Moreover, recent studies show that the basolateral and medial nuclei are involved not only in memory for emotional events, but also in the modulation of memory storage, thanks to its interaction with glucocorticoids (Lupien and McEwen 1997).

It is also well established that the secretion of the end-product of the HPA axis, cortisol, is kept within a narrow range in normal subjects by a complex negative feedback system. The consequence is that excessive activity of this system may result in persistent damage to the organism, affecting the immune, cardiovascular, skeletal, and central nervous systems (Chrousos and Gold 1998; Gold et al 1996). A model integrating genetic and environmental influences is the concept that parental care in early life can regulate the expression of genes in the brain that then determine the nature and magnitude of the animal’s response to stress in adulthood (Caldji et al., 1998).

**HPA AXIS, PSYCHIATRIC DISORDERS AND THEIR MEDICAL CONSEQUENCES**

Increased activity of the HPA system is associated with psychiatric disorders of considerable morbidity, mortality and social cost, such as ano-
rexia nervosa and melancholic depression. On the other hand, atypical depression, Cushing's syndrome, seasonal affective disorder, chronic fatigue syndrome, post-traumatic stress disorder, and hypothyroidism are associated with decreased stress system activity. It is sometimes difficult to distinguish between cause and effect, since this system tends to interact with internal and external perturbations in a "non-specific" manner. Nonetheless, maladaptive responses can act as stressors themselves, sustaining and feeding a vicious cycle. Recent studies (Panarelli et al 1998; Rosmond et al 1998) attempt to demonstrate whether normal-life stress-related hypersecretion of cortisol or hypersensitivity to glucocorticoids may affect systemic blood pressure or carbohydrate and lipid metabolism, leading to deleterious impairments in the long term. Huizenga and colleagues (1998) have recently demonstrated that a polymorphism of the glucocorticoid receptor is present in 6% of normal Dutch men that is associated with a significantly greater cortisol suppression by dexamethasone, a tendency to obesity, and lower bone mineral density. It is possible that a variation in target-gene responsivity to glucocorticoids is the result not only of mutation in the gene of the glucocorticoid receptor, but also in genes that are involved in the glucocorticoid signal-transmission pathway, inducing variations that can be either harmful or protective (Chrousos and Gold 1998). With recent advances in molecular genetics, most of the cytokine and hormone-related genes have been already identified. However, the relation between susceptible genes and phenotype definition has not yet been clarified.

Major depression is a complex disorder associated with a number of hormonal features that may also be risk factors for decreased bone mass density (BMD), including hypercortisolism, hyposomatropism, hypogonadism, and catecholamine hypersecretion (Michelson and Gold 1998). CRH-mediated hypercortisolism is likely to exert more deleterious effects on bone than hypercortisolism of either Cushing's disease or exogenous glucocorticoid administration (Kling et al 1991). The chronic or repeated hyperactivity of the stress system can eventually produce long-term medical morbidity in patients with depression, including effects on bone and the cardiovascular system. A history of melancholic depression is indeed associated with marked osteoporosis in premenopausal women (Michelson et al 1996). Glucocorticoid-induced osteoporosis is characterized by decreased bone formation and in situ death of isolated segments of bone, and is third in frequency after postmenopausal and senile osteoporosis. Recent clinical studies have demonstrated a marked decrease in BMD, as determined by dual energy radiographic absorptometry (DEXA), in women with a history
of depression (Michelson et al 1996). This is of interest from a public health perspective, as major depression affects 5-9% of the general population and it is well known that a decrease in BMD of 10% is associated with an increase in hip fractures at the rate of 40% over a period of 10 years (Michelson et al 1996).

The presence of specific, saturable, high-affinity glucocorticoid receptors (GR), androgen and estrogen receptors has been demonstrated in human osteoblasts. GC are therefore able to influence the production and action of cytokines and growth factors that regulate bone microenvironment. For example, dexamethasone can inhibit both basal and IL-1 mediated interleukin-6 mRNA expression in human osteoblasts (Littlewood et al 1991). Reduced BMD has been found for both male and female patients suffering from major depression; however, males were more severely affected than females (Halbreich et al 1995). Although clinical and experimental findings clearly suggest that patients with mood disorders may be at increased risk of bone fractures, this issue is still widely neglected.

Moreover, patients with depression have a decreased life expectancy primarily due to cardiovascular diseases. Depression has not only been associated with ischaemic heart disease but has also consistently been associated with a worse outcome (Glassman and Shapiro 1998; Musselman et al 1998). It is of note that obesity and myocardial ischaemia are represented in the so-called 'metabolic syndrome', which is characterized by insulin resistance, hypertriglyceridemia, hypertension and reduced fibrinolytic activity (Sirtori and Pasik 1994), and whose pathogenesis involves both hypercortisolism (Pickup et al 1997) and vagal withdrawal (Lee et al 1998). These medical consequences of depression are likely to be the result of dysfunction of neuroendocrine and immune regulation.

In summary, fundamental manifestations of melancholic depression are the hyperarousal and redirection of energy and behaviour typical of the generalized stress response. However, adequate adaptive counterregulation becomes maladaptive and prolonged in melancholic depression. Thus, arousal turns into dysphoric hyperarousal, and vigilance into hypervigilance and insomnia. The dysphoria observed in melancholic depression may represent hyperactivation of the mesocorticolimbic system in response to chronic stress. Patients' cognition is obsessively focused on depressive ideas, impairing their ability to handle practical problems. Furthermore, decreased interest in feeding and reproduction, which is adaptive in generalized stress syndrome, when prolonged can lead to anorexia and absence of libido, which are both hallmarks of melancholic depression. Many other conditions may be
associated with increased and prolonged CRH secretion or activity. Anorexia nervosa, panic disorder, obsessive-compulsive disorder, chronic active alcoholism, alcohol withdrawal, and premenstrual syndrome, all show common manifestations, as hypersecretion of CRH; however other neuroendocrine alterations confer pathophysiological specificity to these disorders. For example, patients with anorexia nervosa have a family history of major depression, are often depressed themselves and also present immune alterations typical of major depression (Licinio et al 1996; Staurenghi et al 1997). On the other hand, they show neural mechanisms of hunger and satiety, as hypersecretion of arginine vasopressin, that could direct them towards pathological eating behaviour (Gold et al 1996). The theory that depression is associated with hyperactivity of CRH neurons could not at first be reconciled with atypical depression and Cushing’s syndrome, in which polyphagia, weight gain and hypersomnia contradict CRH hypersecretion. Indeed, a variety of studies suggested that CRH secretion was decreased in these patients (Gold et al 1988b). Studies in seasonal affective disorder showed chronically decreased CRH secretion in the winter depressed state (Gold et al 1995; Joseph et al 1991), together with higher plasma cortisol sensitivity to glucocorticoids, but cortisol production rate is reduced (Walker et al 1997). It has therefore been proposed, based on those data, that there is another form of stress system dysregulation characterized by hypoarousal of the CRH system. This category could include some forms of obesity characterized by a hypoactive, hyposerotonergic HPA axis (Bernini et al 1989), although we must take into account the effect of leptin, a hormone secreted by fat cells, on modulating the magnitude of CRH release (Licinio et al 1997; Prolo et al 1998). Subgroups of patients with post-traumatic stress disorder have shown decreased urinary free cortisol secretion and increased sympathetic system activity in reaction to memories of past stressors (McFall et al 1990; Yehuda et al 1990). Recent research, however, contradicts these findings (Laudenslager et al 1998).

The cloning of CRH receptors and their localization in the brain have led to the development of receptor antagonists of potential use in the treatment of melancholic depression and other conditions associated with high CRH bioactivity. Antalarmin is a novel CRHRI antagonist, a pyrrolopyrimidine (non-peptide) compound, that blocks the effect of CRH after peripheral administration (Deak et al 1999). A very recent study in the rat (Wong 1999) has demonstrated that antalarmin administration for 8 weeks blunts basal HPA function and decreases basal adrenal response to ACTH, but it does not affect the adrenal response to an acute stressor. Future clinical studies on humans of chronic
treatment of diseases associated with high CRH activity, such as major depression, are needed and should consider both basal and stress-stimulated HPA function.

**HPA AXIS AND THE ADIPOCYTE HORMONE LEPTIN**

Leptin was discovered in 1994 by Zhang et al as the cytokine product of the *ob* gene (Zhang et al 1994). Absence of leptin is responsible for the obese phenotype of ob/ob mice. For this reason it has since received considerable attention for its potential use in the treatment of human obesity and as a clue in the study of weight-related disorders such as anorexia nervosa. The primary role of leptin is to provide information to hypothalamic areas about energy homeostasis, thus acting as an important controller of the size of fat stores by lowering food intake once released into the circulation. Recently our group (Licinio et al 1997) and others (Sinha et al 1996) have demonstrated that plasma levels of leptin are pulsatile and present diurnal variations, and are inversely correlated with cortisol concentrations.

Leptin communicates the metabolic status of the peripheral adipocyte to the brain. In animal and human studies, weight loss results in a decrease in leptin, while weight gain significantly increases circulating leptin. However, recent research suggests that regulation of leptin is more complicated than just stated, and it responds to caloric restriction and to reduction in adipose tissue masses as well (Considine et al 1996).

The first question asked since the identification of the *ob* gene has been about the role of leptin in the development of obesity. The relationship between leptin and obesity has been shown by single gene defect animal models of obesity. Recently, congenital leptin deficiency was reported as related to morbid obesity in humans too (Montague et al 1997). Since plasma leptin levels are highest between midnight and the early morning hours and lowest from noon to mid-afternoon, the nocturnal rise has been associated with the suppression of appetite during sleep and the lower daytime concentrations with increased energy expenditure (Matkovic et al 1997). Leptin concentrations and the HPA axis are closely linked in an inverse relationship (Licinio et al 1997). Leptin is also a trophic factor for the reproductive system, and a dynamic relation between leptin and the hypothalamic-pituitary-ovarian (HPO) axis hormones is conceivable. Leptin seems to affect reproduction at various levels by acting directly on the hypothalamus, pituitary gland, and ovary. Rhythmic patterns of LH and leptin in healthy women in the mid to late follicular phase of their menstrual cycle are significantly different from random. Moreover, a nocturnal
rise in plasma leptin seems to be associated with a great change in the pattern of LH pulses – from rapid and smaller pulses during the day to fewer and longer during the nocturnal rise of leptin (Licinio et al. 1998). Such a close association between leptin and LH may provide an additional level of communication between nutritional status and the reproductive axis. A recent study in the rat brain has shown that leptin administration activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei, thus providing an anatomical substrate for the integration of metabolic and circadian information to regulate the hypothalamic-pituitary axis (Elmqquist et al. 1998). Leptin thus affects feeding, metabolism, and neuroendocrine status. Moreover, the leptin-hypothalamic-pituitary-ovary and HPA axis interactions may be of clinical importance. Evidence that leptin plays an important role in the reproductive function gave rise to the hypothesis that low leptin synthesis is associated with amenorrhea. However, studies in women with functional hypothalamic amenorrhea, bulimia and anorexia nervosa showed that a critical leptin level is needed to maintain menstruation (Kopp et al. 1997; Laughlin and Yen 1997).

The role of altered leptin concentrations in the pathophysiology of ovarian disease has also been proposed. Some authors studied the effect of leptin in polycystic ovarian syndrome, but results are controversial and the expected elevation of leptin levels were not found (Laughlin et al. 1997; Mantzoros et al. 1997b). Subjects with Cushing’s disease showed elevated body-mass index (BMI) and plasma leptin levels compared to controls, which remained unchanged after successful transphenoidal surgery and directly proportional to BMI. Despite massive elevations in plasma ACTH and cortisol, plasma leptin levels were not affected by CRH infusion either in patients or in controls (Cizza et al. 1997).

More recent findings have led to the suggestion that leptin may be involved in the cytokine-induced anorexia wasting syndrome of infection. Leptin was shown to be three-fold higher in patients who survived a septic episode than in non-survivors (Bornstein et al. 1998). On the other hand, subjects with anorexia nervosa have decreased leptin levels (Mantzoros et al. 1997a). Since changes in food consumption do not affect leptin levels, the primary role of leptin in critically ill subjects is not the prevention of obesity, but rather represents an acute stress-mediated response. Leptin has not only an adipostatic function but also appears as a stress-related hormone, which might be important to survival. The relevance of leptin in various other stress- or eating-related human pathologies is currently under investigation.
HPA AXIS AND THE IMMUNE SYSTEM

The hypothalamic-pituitary-adrenal (HPA) axis has been the focus of extensive research because of its important role in maintaining physiological homoeostasis under both basal and challenge conditions (Licinio 1998b). The HPA axis and the immune system participate in a key regulatory cascade. In fact, these two systems do not function independently: hormones are potent modulators of immune function (for instance, glucocorticoids suppress interleukin-2 but enhance interleukin-4 activity) and, conversely, immune cells are capable of synthesizing hormone-like signals (Sternberg and Licinio 1995).

Cytokines are a large family of polypeptide mediators classically associated with the regulation of immunity and inflammation. The major proinflammatory cytokines, interleukin-1 (IL-1) and tumour necrosis factor alpha (TNFα), together with interleukin-6 (IL-6), not only regulate the inflammatory process at the local tissue level, but also influence a variety of CNS-mediated responses to infection and inflammation, such as fever, sickness behaviour, and changes in neuroendocrine activity (Sternberg et al 1992). The most significant, and certainly the best studied, of neuroendocrine responses to inflammatory insults, is activation of the HPA axis (Licinio and Wong 1997), leading to the concept of a hypothalamic-pituitary-adrenal-cytokine circuit operating in both basal and challenge conditions.

Natural killer (NK) cells are important targets of endocrine-immune interaction. NK cells are a CD8+, TCR subset, that are spontaneously cytotoxic against susceptible targets as tumour-transformed or virus-infected cells. NK cell activity is sensitive both in vivo and in vitro to cytokines and HPA axis hormones. Glucocorticoids are potent in vitro negative modulators of human NK cell activity, which is also modulated by at least two proopiomelanocortin (POMC)-derived peptides, ACTH and β-endorphin (Gatti 1993; Sternberg and Licinio 1995). Thus, immunosuppressive effects of glucocorticoids may be counterbalanced by POMC-derived peptides. Inverse relations between spontaneous NK cell activity and severity of symptoms have already been described in major depression (Nerozzi 1989) although no distinction between typical and atypical features has ever been considered. The issue of whether disturbances of NK cell activity in humans may be an epiphenomenon of stress is still debated. Angeli et al (1992) did not find statistically defined abnormalities of spontaneous NK cell activity either in subjects with anorexia nervosa (Staurenghi et al 1997) or in patients with Cushing’s syndrome (Masera et al, in press), while subjects at the early onset of Alzheimer’s disease (AD) showed enhanced levels of NK activity in comparison to an age-and sex-matched control
group that were very similar to those from a group of younger normal volunteers; however, in vitro response of NK cells to cortisol at physiological concentrations was significantly related to the severity of cognitive symptoms in AD (Prolo et al, unpublished data).

The brain and the immune system can either stimulate or inhibit each other. Cytokines stimulate CRH secretion in the hypothalamus. CRH in turn activates HPA axis hormone production. The final product of the HPA axis, cortisol, then suppresses the immune system. CRH can also activate the SAM axis, thereby modulating heart rate, blood pressure, and inflammatory responses. Sternberg and co-workers have shown that a disruption of any component of this circuit results in alteration in susceptibility to inflammation and autoimmunity (Sternberg et al 1989a; 1989b).

Immune activation also causes production of nitric oxide (NO). Interleukin-1β is a key factor in the transcription and translation of the inducible nitric oxide synthase (iNOS) gene. Nitric oxide has a variety of effects on neuroendocrine regulation, particularly during the response to inflammatory stressors.

**HPA AXIS AND NITRIC OXIDE**

Nitric oxide (NO) is a labile free radical gas, whose pivotal role in brain injury is now well established, but it has long been speculated that its toxicity may be the price paid for equipping cells with the means to overcome damage due to infections or other injuries (Licinio et al 1999; Murad 1998). NO released during inflammatory stress can diffuse into neuropeptide-secreting neurons and modulate neuroendocrine function and possibly behaviour during sepsis. For example, NO can modulate the release of corticotropin-releasing hormone (CRH) and luteinizing hormone-releasing hormone (LHRH) from rat hypothalamus (McCann 1997; Rettori et al 1994). Neuroendocrine changes elicited by systemic inflammation include activation of the HPA axis, the sick euthyroid syndrome, decrease in growth hormone (GH) activity, and cessation of reproductive function (Dennhardt et al 1989). These are associated with behavioural changes such as fatigue and lethargy. Several groups worldwide are currently investigating whether NO stimulates or inhibits specific brain functions, and it seems that effects of NO on specific brain functions may be, at least in part, dependent on the dose of NO at a particular site.

Recent research on brain ischaemia has indicated that competitive NOS inhibitors, such as NG-nitro-L-arginine methyl ester (L-NAME) (Merrill and Murphy 1997), can rescue neurons from death through the inhibition of iNOS activity. However, NO produced by microglial
cells during acute brain injury in rats causes damaged neurons to undergo apoptosis; this actually delays neuronal death in the area surrounding the injury, by eliminating damaged neurons next to the necrotic area. This process may reintegrate neuronal circuits with undamaged and functionally intact neurons. Moreover, iNOS has been identified in remyelinating lesions of the spinal cord after lysolethicin-induced demyelination (Merrill and Murphy 1997), and is therefore thought to have a role in remyelination, possibly by promoting gene expression and by affecting signalling pathways. The molecular mechanism for the effects of NO on repair appear to involve the expression of genes encoding phosphatases, tyrosine kinases, serine-threonine kinases, and the heme-regulated eIF-2 kinase. Redox-regulated transcription factors and activators include p53, AP-1, NF-kB, Ets, Sp-1, glucocorticoid receptor, and Egr-1, and the immediate early genes c-fos and c-jun. NO also increases TNF-a, IL-6 and IL-1B gene expression in vivo and in vitro (Merrill and Murphy 1997). The role of iNOS in brain repair is a highly promising area of active investigation at the present time.

CONCLUSIONS
Both chronic and acute behavioural stress are associated with transient or chronic alterations in the concentrations of many neuroendocrine effectors that might be related to a characteristic phenotype. For instance, in addition to risky health behaviours such as smoking, increased alcohol consumption, and dysregulated eating, several biological characteristics of persons with trait hostility (Brummet et al 1998) have been identified that could account for increased rates of severe pathologies: increased cardiovascular reactivity to stress, increased sympathetic activation, decreased parasympathetic function, increased HPA axis reactivity, and altered function of cells of the immune system (chiefly the monocyte/macrophage component). This clustering is mediated by functional alterations in brain circuitries as a result of genetic and environmental (especially decreased nurturing and increased adversity in early childhood) influences.

It is noteworthy that neuroendocrine systems show either a circadian organization or one dependent on the rest-activity/sleep-wake cycle. Immune effectors appear to be regulated in the same way (Angeli et al 1992). These temporal organizations may have functional implications. For example, ACTH and cortisol levels correlate directly with the active period both in diurnal and nocturnal animals, indicating that higher levels of adrenal corticosteroids are necessary for the awake state. In contrast, cerebrospinal fluid levels of CRH present diurnal variations, with higher levels in the evening and lowest levels early in the
morniing, that are the opposite of plasma cortisol levels, which show a peak around 08:00 and a nadir around 22:00 (Kling et al 1994). Unfortunately, most studies have considered only few time points, usually in the morning. In order to fully understand human neuroendocrine function, detailed studies are required with as many evaluation points as possible, under rigorous study conditions (Gold et al 1996; Licinio and Gold 1997). After data are collected it is critical to not only examine plasma hormone concentrations and (through the use of deconvolution methods) secretion rates, but also to assess sequence-dependent patterns, by assessment of parameters such as approximate entropy (ApEn), which quantifies the orderliness of sequential measures (Pincus and Huang 1992) such as hormonal time series.

It seems reasonable to conclude that the neuroendocrine and behavioural responses to different stimuli are strongly but not solely dependent on genetic factors. For instance, like humans, rats inhabit a tremendous variety of ecological niches, each with distinct environmental demands. Behavioural and neuroendocrine responses thus reflect a naturally occurring plasticity, where the behaviour of the mother results in the programming of various responses to threatening stimuli. Because most mammals, as did humans in the past, spend their adult life in either the same or in a very similar environment to that in which they were born, "programming" of central nervous system response to outside stimuli in early life is of adaptive value to the adult, avoiding the need for a longer and perhaps unaffordable period of adaptation or learning in adult life. The mother's behaviour is therefore a link between pups and habitat, and serves as a source of information for the correct functioning of neural circuitries that regulate endocrine and behavioural reactions to stress (Caldji et al 1998).

Gene expression is affected by the environment and, together, genes and psychosocial factors determine behaviour. Forty years after his first paper (Selye 1976), H. Selye stated about the stress of living that: "Love thy neighbour as thyself" can be translated as "Earn thy neighbour's love". This attitude will best ensure homeostasis and resistance to stressors throughout life and give a satisfactory purpose to one's activities.

REFERENCES


Molecular neuroendocrinology and its impact on behaviour


Genes, behaviour and health
CYTOKINE GENE EXPRESSION IN THE BRAIN: IMPACT ON SICKNESS BEHAVIOUR

Ma-Li Wong

ABSTRACT
The pathophysiology of systemic inflammation involves peripheral organs such as the kidney, heart and gut, as well as the brain, affecting temperature regulation, sleep and behaviour. Cytokines can act in the brain through one or more of the following mechanisms: (1) disruption of the brain-blood-barrier (BBB), (2) penetration in the brain through circumventricular organs, (3) de novo synthesis in the CNS, (4) action in peripheral nerves which signal the brain. To date several cytokines and their receptors have been found in resident central nervous system (CNS) cells, both in neurons and glia. In the CNS, cytokine genes can be expressed under resting physiological conditions, but they can also be induced during pathophysiological states in the brain and in the periphery. The hallmarks of cytokine biology are pleiotropism, redundancy, and feedback. The specific actions of each individual cytokine are somewhat concealed by the fact that many of their actions overlap and because cytokines regulate one another to form a cytokine network. The CNS responds to systemic inflammation with robust interleukin 1 beta (IL-1β) gene expression and limited counter-regulation by IL-1 receptor antagonist (IL-1ra), IL-10, and IL-13 gene expression; in contrast the response of the periphery to systemic inflammation is predominantly counter-regulatory. This chapter discusses the emerging concept of central and peripheral cytokine compartments, and the evidence that led us to conceptualize that these two compartments are differentially regulated, but integrated in a complex network of central and peripheral cytokines.

INTRODUCTION
IL-1 action is regulated by a complex network of molecules that includes several ligands [IL-1α, IL-1β (1, 2), and IL-1 receptor antagonist (IL-1ra)] (3, 4), multiple binding sites [IL-1 receptor type I (IL-1RI) (5), IL-1RII (6), IL-1 accessory protein (7), soluble receptors (8),
receptor related proteins (9), receptor like molecules (10), and autoantibodies], and a key regulatory enzyme [IL-1β converting enzyme (ICE) or caspase 1] [for a review see reference (11)].

Brain cells, including neurons, microglia, endothelial cells and astrocytes can produce interleukin 1 beta (IL-1β) in response to various physiological and pathological stimuli. The typical CNS action of the prototypical cytokine IL-1 is fever, but cytokines have also been reported to influence several CNS functions such as sleep, food intake, cognition, behaviour, and neuroendocrine regulation. Several lines of research suggest that endogenous IL-1 can also mediate neurodegeneration in the rat brain, although the pathways and mechanisms for cytokine signalling of the CNS remain to be fully elucidated.

IL-1 can also activate the BBB (12), elicit the release of arachidonic acid, NO, and β amyloid precursor protein. IL-1β immunoreactivity has been shown to be increased in brain perivascular leukocytes and parenchymal microglial cells in brain tissue of patients with AIDS encephalitis (13, 14). ICE can also be involved in neuronal cell death (15). Similarly, neurotrophic and protective effects of cytokines in brain neurons have been clearly demonstrated. Increased brain cytokine expression has been observed in many neurological disorders, including Alzheimer's disease, Down's syndrome, multiple sclerosis, temporal lobe epilepsy, Parkinson's disease and human immunodeficiency virus infections (16-18). Thus, depending on amount and site of expression IL-1β can be either neuroprotective or neurotoxic. Available evidence indicates that in high levels, in the context of acute events, IL-1β is neurotoxic. It is possible that the actions of IL-1 are also mediated through CRH in extra-hypothalamic sites; in fact recent data in models of acute brain ischaemia suggest that local increases in CRH contribute to acute neurodegenerative processes, while IL-1ra (an endogenous IL-1β antagonist) synthesis is involved in the process of neuroprotection.

CENTRAL CYTOKINE RESPONSE IN INFLAMMATION

Profound signs and symptoms mediated by the central nervous system (CNS) are caused by inflammation originating from peripheral sites. This is reflected in our language: we say that we feel ill when we refer to the CNS manifestations of peripheral inflammation. Those manifestations include alterations in temperature regulation and cognition, suppression of locomotion and exploration, reductions of food intake and sexual behaviour, and increase in sleep and lethargy (19). These CNS manifestations of peripheral inflammation have been proposed to be mediated by brain IL-1β synthesized during systemic inflammation.
CNS signs and symptoms of systemic illness can be reproduced by central exogenous IL-1β administration and prevented if high levels of IL-1ra are administered in conjunction with IL-1β centrally (19). Furthermore, IL-1β knockout mice have no fever and no alterations in ingestive behaviour in response to peripheral inflammation (20). The CNS response to infection can include marked induction of cytokine production in brain parenchyma. During infections, bacterial products, such as lipopolysaccharide (LPS), cause the release of cytokines from immune, endothelium (21) and brain cells (22). LPS binds to its receptors on these cells, resulting in the release of various cytokines, such as interleukin-1β (IL-1β), TNF-α, IL-6, IL-2, and γ-IFN. The pattern of release of cytokines depends on the type and severity of infection (23). Cytokine signalling and pathways in the CNS pathways include: direct transport into the CNS, action at peripheral sites, such as neural-associated lymphoid tissue, actions at the level of brain vasculature, and direct entry into CNS areas lacking blood-brain barrier (circumventricular organs, which include the area postrema, pineal gland, subcommissural organ, subfornical organ, median eminence, neurohypophysis, and organum vasculosum of the lamina terminalis) (24). Injection of moderate amounts of LPS mimic the effect of bacterial infection, increasing IL-1β in the paraventricular nucleus, arcuate nucleus, median eminence, choroid plexus, meninges, and the anterior pituitary and pineal.

**HIGH IL-1β/IL-1ra mRNA IN THE BRAIN**

The biological effects of IL-1 are a reflection of the local ratio of IL-1β and of cytokines that inhibit IL-1 action (24). During systemic inflammation CNS manifestations of peripheral inflammation are mediated by IL-1β synthesized de novo within the brain with limited synthesis of counter-regulatory cytokines, such as IL-1ra, and IL-10. The induction of IL-1β gene expression in the CNS during systemic inflammation indicates that IL-1β may be an important and multisite neuroregulator of the metabolic adaptations to inflammatory stressors. The findings that exogenous IL-1β administration causes the characteristic behavioural symptoms associated with peripheral illness, and that those behavioural effects of IL-1β are inhibited by exogenous central IL-1ra administration (25-28), support the notion that the actions of brain IL-1β are so evident during systemic inflammation because there is only very limited cytokine counter-regulation in the brain. Thus, in the brain, antagonism or knock-out of IL-1β can abolish sickness-associated behavioural signs and symptoms caused by IL-1β. In the periphery, IL-1 induction is followed by a robust response of IL-1ra,
IL-10, or IL-13, cytokines that inhibit IL-1 synthesis and function. Granowitz et al (29) reported that the peripheral IL-1ra response to LPS is 100-fold greater than that of IL-1β. However, in contrast to the periphery, the brain response to endogenous IL-1β induction does not result in marked induction of IL-1ra (22).

**IL-1ra IS AN ENDOGENOUS NEUROPROTECTIVE MOLECULE**

IL-1ra is a naturally occurring endogenous antagonist of IL-1 action; it binds to IL-1 receptors without inducing signal transduction; hence, IL-1ra is a pure antagonist, with no partial agonist actions. IL-1ra acts at the local levels and it also enters the bloodstream to target distant sites. In a model of focal cerebral ischaemia in rats, Loddick et al (30) showed that the inhibition of the action of IL-1ra by immunoneutralization markedly enhances ischaemic damage in the brain; therefore, IL-1ra can act as an endogenous neuroprotective agent.

**DIFFERENTIAL REGULATION OF CENTRAL AND PERIPHERAL CYTOKINE COMPARTMENTS**

Indeed in the CNS, cytokine counter-regulation studied by the expression of three genes encoding different cytokines that inhibit IL-1β bioactivity, namely IL-1ra, IL-10 and IL-13, was found to be limited (22). IL-10 is known to inhibit IL-1β bioactivity, IL-13 counter-regulates IL-1β bioactivity by inhibiting the synthesis of IL-1 and by inducing the synthesis of IL-1ra and of the type II IL-1 receptor (an endogenous decoy for bioactive IL-1).

Those differential patterns of central and peripheral IL-1β counter-regulation can be illustrated by findings in the pituitary gland. Figure 1 illustrates the findings in the anterior and posterior pituitary. Embryologically and anatomically the posterior pituitary is part of the CNS, while the anterior pituitary is a peripheral organ. Thus, in the posterior pituitary high levels of IL-1β mRNA induction were found, with a limited IL-1ra mRNA response, which is the same pattern that we observed throughout the CNS. In contrast, the anterior pituitary displayed a pattern of IL-1β induction that has been previously described in the periphery (29), characterized by increases in IL-1ra gene expression that far exceed the local induction of IL-1β mRNA because local levels of IL-1ra must be several-fold higher than those of IL-1β to effectively modulate IL-1 bioactivity (24).

During illness it is advantageous to counter-regulate IL-1 action in the periphery, thus limiting inflammation. On the other hand the actions of IL-1 in the brain may be advantageous, as those actions cause one to feel ill, leading to sleep, decreased search for food and reproduc-
tion, and suppression of locomotion and exploration, thereby facilitating recovery. The differential patterns of IL-1β counter-regulation led us to conceptualize that there are two cytokine compartments, central and peripheral, and that these two compartments are integrated but differentially regulated. The integration of the responses of the central and peripheral cytokine compartments would be essential for the successful resolution of any systemic inflammatory state.

CENTRAL iNOS RESPONSE IN INFLAMMATION
The limited counter-regulation of IL-1β actions in the CNS is reflected by the robust induction of central inducible nitric oxide synthase (iNOS) (31). IL-1β is a potent stimulus to iNOS transcription and activity. IL-1 has been shown to induce iNOS mRNA and NO production in vascular and brain cells in vitro (32) and to induce iNOS in vivo. The effects of IL-1 on inducible iNOS mRNA are stimulated by tyrosine kinase, potentiated by eicosapentaenoic acid, and inhibited by angiotensin II, actinomycin D, cyclohexamide, transforming growth factor-beta 1, and insulin-like growth factor I. Based on the findings of high IL-1β/IL-1ra mRNA in the brain during systemic inflammation, we conducted experiments to test the hypothesis that pathophysiologically significant iNOS gene transcription and bioactivity was induced in the brain during systemic inflammation. At baseline we found detectable iNOS gene expression in the brain, but a detailed neuroanatomical study revealed that early in the course of systemic inflammation there is a profound induction of iNOS mRNA in vascular, glial, and neuronal structures of the rat brain (33). Two neuronal hypothalamic nuclei showed strikingly high induction of iNOS mRNA: PVN and the arcuate nucleus. iNOS mRNA levels were also markedly induced in both endocrine glands that are situated in close proximity to the brain, the pituitary and the pineal.

During systemic inflammation, iNOS gene expression was accompanied by the production of nitric oxide (NO) metabolites in brain parenchyma and cerebrospinal fluid (CSF).

NO is involved in important physiological functions of the CNS, including neurotransmission, memory and synaptic plasticity. Depending on the redox state of NO, it can have neurotoxic or neuroprotective actions. It has been suggested that NO may play a role in the pathogenesis of neurodegenerative disorders such as Parkinson’s disease, Alzheimer’s disease and Huntington’s disease. NO at high levels is known to be cytotoxic, and may be involved in a range of inflammatory, neurodegenerative, and cardiovascular/ischaemic pathologies. The mechanism of NO-induced cytotoxicity is not clearly understood; nanomolar
levels of NO reversibly inhibit mitochondrial respiration by competing with the oxygen binding site of cytochrome oxidase. The inhibition of oxygen consumption by NO has been reported in mitochondria, brain nerve terminals, and cultured cells.

During systemic inflammation, the spill-over of NO metabolites into the CSF has the potential to be a diagnostic marker for systemic inflammation and sepsis. Therefore, the metabolic by-products of cytokine action within the brain may be of diagnostic relevance in human disease.

INTEGRATION OF CENTRAL AND PERIPHERAL CYTOKINE COMPARTMENTS

Immune system-brain communication
Analysis of the temporal and spatial patterns of IL-1β, IL-1RI, and iNOS gene expression in vascular and perivascular areas of the rat brain during the course of LPS-induced systemic inflammation (21) has led to the hypothesis that IL-1β-iNOS interactions in brain vasculature were a mechanism for immune system-brain communication. IL-1RI is constitutively expressed at the interface of the vascular wall and perivascular glia. During systemic inflammation, vascular IL-1β, binding to vascular and perivascular IL-1RI receptors, may induce perivascular iNOS gene expression, leading to the production of NO and modulation of the effects of IL-1β in the brain. We have postulated that the vascular and perivascular induction of iNOS mRNA by IL-1β may represent a mechanism for the modulation of the CNS effects of peripheral inflammatory mediators (34). It is very likely that other barrier-related sites can also contribute to the actions of cytokines in the CNS (34, 35). Given that the hallmarks of cytokine biology are pleiotropy and redundancy, it is not surprising that there seem to be redundant pathways by which circulating IL-1β acting on vascular and perivascular IL-1 type I receptors signals the brain. In addition to iNOS induction, vascular IL-1RI activation causes prostaglandin synthesis (35). These different lines of evidence seem to indicate that during peripheral inflammation IL-1 acts within the brain to mediate sickness behaviours induced by peripheral cytokines. However, because the signal-transducing type I IL-1 receptor has not been identified in brain areas whose activities are modulated by IL-1β, it is difficult to explain how central IL-1β might mediate these effects at the cellular and molecular levels. Sawchenko and his group have shown that IL-1β binding to vascular IL-1RI activates via a prostanoid pathway: medullary aminergic neurons that communicate directly with the PVN and signal CRH-producing neurons (36).
In response to peripheral inflammation, peripheral cytokines such as IL-1β are synthesized and may circulate in the bloodstream. This is followed by the synthesis of IL-1β in key areas of the brain, such as the PVN, an area that is well known to respond to that cytokine; however, IL-1 receptors do not seem to be present in those areas. One plausible explanation for this apparent paradox would be that IL-1β acting in areas that send inputs to the PVN, such as the medulla, areas that are adjacent to the PVN, or at the level of brain vasculature (21) within the PVN, might result in the generation of other informational molecules, such as prostanooids (35, 37) or NO (38), that would modulate the effects of IL-1 on PVN function.

**Brain-immune system communication**

The functioning of the CNS and periphery during systemic inflammation is modulated not only by cytokines synthesized from peripheral sites, but also by cytokines that are generated by the CNS. We have already discussed that during systemic inflammation key areas that drain to the anterior pituitary, such as PVN, arcuate nucleus, median eminence, and posterior pituitary, all express high levels of IL-1β mRNA; we have also discussed that the anterior pituitary expresses moderate levels of IL-1β followed by very high levels of expression of the mRNA for the secreted isoform of IL-1ra, which is known to be induced by IL-1β.

IL-1β synthesized by the PVN could be secreted into hypophyscal portal blood as a hypothalamic neurohormone that regulates pituitary function. During inflammation IL-1β achieves levels in the circulation that can directly stimulate pituitary cells to secrete ACTH, LH, GH, and TSH, and inhibit the secretion of prolactin (39). These pituitary effects have led Bernton and colleagues to propose that IL-1, acting directly at sites such as the pituitary gland, may be an important regulator of the metabolic adaptations to infectious stressors.

We therefore propose that the pituitary secretion of IL-1ra may represent not only a novel systemic hormonal anti-inflammatory mechanism but also a pathway of central-to-periphery integration that is elicited during systemic inflammation by IL-1β that reaches the anterior pituitary originating from multiple sources that include PVN, arcuate nucleus, median eminence, posterior pituitary, systemic circulation, and the anterior pituitary itself (22). Recently, direct secretion of IL-6 from the brain has also been postulated to be a pathway of neuro-immunomodulation. Reichlin and colleagues (40) have shown that the brain and its supporting structures are activated by intracerebroventricular IL-1β to release IL-6 into the blood and that such an effect
is independent of peripheral sympathetic activity or central mobilization of CRH.

Therefore, the cross-talk between peripheral and central cytokine compartments is such that peripheral cytokines, synthesized in immune cells, affect the functioning of the brain, and central cytokines may be secreted by the brain to modulate peripheral immune function.

CONCLUSION

In summary, central manifestations of peripheral inflammation are mediated by endogenous brain IL-1β synthesized during systemic inflammation in the context of limited central cytokine counter-regulation of IL-1, while in the periphery cytokine counter-regulatory response is the predominant one; evolutionary forces may have influenced the different pattern of central IL-1 counter-regulation. One could postulate that during systemic inflammation it could be advantageous to counter-regulate IL-1 action in peripheral body fluids, thus limiting the inflammatory response, but it may be advantageous not to counter-regulate the actions of IL-1 in the brain, as those actions cause sickness behaviour that could facilitate recovery.

We believe that the integration and differential regulation of central and peripheral cytokine genes is a key element for the optimal functioning of the immune and nervous systems.
FIGURE 1
Induction of IL-1β and IL-1ra mRNAs in the anterior and posterior pituitary after LPS treatment. Computer-generated pseudocolour images for the peak mRNA induction are shown in A for IL-1β (2 h after LPS administration) and in B for IL-1ra (6 h after LPS administration). IL-1β mRNA induction occurs predominantly in the posterior pituitary (A) and IL-1ra mRNA induction occurs predominantly in the anterior pituitary (B). (C) Picture of gel with RT-PCR product of RNA obtained from pituitaries of rats treated with LPS, 6 h after injection. A single band of the expected size for secretory IL-1ra (~537 bp) was found; cloning and sequencing of the RT-PCR product confirmed the specific sequence of secretory IL-1ra. (D) High magnification image of IL-1ra mRNA hybridization in the anterior pituitary; silver grains overlying IL-1ra mRNA is shown as black dots. (E and F) Graphics show the time course for the induction of IL-1β mRNA (red line) and IL-1ra mRNA (blue line) in the anterior pituitary (E) and in the posterior pituitary (F) at 0, 2, 6, and 24 h after LPS administration, using quantitative densitometry from autoradiographic images. Anterior and posterior pituitary increases in mRNA levels over time-matched control values were significant for IL-1β at the 0.0001 level at 2 h and at the 0.05 level at 6 and 24 h, and for IL-1ra at the 0.05 level at 2 h and at the 0.0001 level at 6 h (ANOVA with post hoc correction). Colour scale for A and B: black indicates background and red indicates areas of highest hybridization levels. Bar = 5μm in D. [Reproduced from Ref. 22 (Proc Natl Acad Sci USA, 1997, 94, 227-232) with permission]
REFERENCES


DOPAMINE D4 RECEPTOR, PERSONALITY AND SUBSTANCE ABUSE

Richard P. Ebstein and Moshe Kotler

PERSONALITY

Personality and temperament (Loehlin, 1992) refer to the characteristic manner or style of an individual’s behaviour, as distinct from the goals towards which it is directed (motivation), or the machinery of its execution (cognitive and motor skills). Temperament and personality may refer to purely stylistic features of behaviour, such as the vigour, tempo or persistence with which it is carried out, or to the emotional expression that accompanies it, such as fearfulness, exuberance, aggressiveness, or self-restraint. The features of behaviour so designated must be typical or enduring and characteristic of a person over a reasonably extended period of time if it is to be called personality or temperament.

Self-report questionnaires developed by psychologists over the past three decades often identify similar personality factors. Of particular interest is a specific personality factor (Novelty or Sensation Seeking) which reasonably correlates between tests including Cloninger’s Novelty Seeking (Cloninger, 1987), Zuckerman’s Impulsivity-Sensation Seeking (Zuckerman, 1994) Eysenck’s Psychoticism & Extraversion (Eysenck and Eysenck, 1978; Zuckerman, 1994) and some of the Big Five factors (Conscientiousness) (Bergeman et al., 1993). This personality dimension is often associated with maladaptive behaviours, especially substance abuse and aggression.

Novelty Seeking is one of four temperament factors defined by Cloninger’s Tridimensional Personality Questionnaire (Cloninger, 1987). The TPQ discriminates between four temperament traits: Novelty Seeking (exploratory, curious, impulsive, extravagant), Harm Avoidance (worrying and pessimistic, fearful, shy, fatigable), Reward Dependence (sentimental, dedicated, dependent) and Persistence (industrious, hard-working, ambitious, perfectionist).

The Novelty Seeking trait comprises elements of impulsivity, extraversion, exploratory activity, curiosity, disorderliness and correlates
highly with Zuckerman’s Sensation Seeking dimension. Alcoholics are distinguished by high Novelty Seeking scores, especially Cloninger’s type II variety characterized by early onset and family history (Bulik et al., 1994; Wills et al., 1994). Novelty seeking also figures prominently in other varieties of substance abuse including opioid abuse (Vukov et al., 1995) and cigarette smoking (Pomerleau et al., 1992). We have observed that Harm Avoidance (independent samples t test; t=4.99, P<0.001) and one sub-scale of the Novelty Seeking dimension, NS3 (extravagance) (t=4.97, P<0.001), were significantly higher in a group of heroin addicts (Table 1) (Mel et al., 1998). The marked increase in Harm Avoidance scores for these subjects may reflect the state sensitivity of this factor, since these addicts were in a therapeutic community rehabilitation programme requiring abstinence from all drugs.

Similar relationships were observed when investigators employed Eysenck’s Personality Questionnaire. Smoking was associated with Extraversion and Psychoticism (Arai et al., 1997; Barrett et al., 1996). Heightened impulsivity and extraversion is associated with degree of severity of psychological and behavioural change in pathological gambling (Blaszczynski et al., 1997). Alcoholics score higher on Eysenck’s Psychoticism scale (Cruz et al., 1995; King et al., 1995). Not surprisingly, abstinence after one year corresponded negatively with impulsivity in alcoholics (McCown, 1990). Again, risky driving correlates with Extraversion and Psychoticism (Martin and Boomsma, 1989). Finally, factor analysis of Eysenck’s psychoticism dimension demonstrated that impulsiveness is an important trait in high Psychoticism (P) scorers (Howarth, 1986).

It should not be overlooked that Novelty or Sensation Seeking, similar to the other TPQ temperament factors, is also part of the normal repertoire of human personality with many positive facets. For example, Air Force pilot recruits had higher scores in sensation-seeking-related scales, suggesting disinhibited behaviour in the social sphere, interest in sports and activities involving some danger, and a need for change. They also had higher scores on an impulsivity scale that comprises sensation-seeking content (Klinteberg et al., 1992). Not surprisingly, similar high sensation seeking has been observed in people engaged in some sports such as bungy jumping (Michel et al., 1997) and sky diving (Hymbaugh and Garrett, 1974).

A number of twin studies (Bergeman, et al., 1993; Heath et al., 1994; Hur and Bouchard, 1997; Loehlin, 1992; Macaskill et al., 1994; Zuckerman, 1994) demonstrate that normal personality traits, as measured by dimensional scales of personality assessment, are partially inherited and 30-60% of the observed variance can be accounted for by
genes. Although the evidence is strongly supportive of a substantial role of inheritance in the determination of personality and temperament, very little is known of the number or nature of the responsible genes.

**DOPAMINE D4 RECEPTOR**

Five members of the dopamine receptor family have so far been identified (Missale et al., 1998). Two D1-like receptor subtypes (D1 and D5) couple to the G proteinGs and activate adenyl cyclase. The other three receptors (D2, D3, and D4) also couple to G proteins but inhibit adenyl cyclase and activate K+ channels. Dopamine receptors are widely expressed in the CNS and are involved in the control of locomotion, cognition, emotion, affect, neuroendocrine secretion and the mechanism of brain reward-reinforcement.

The dopamine D4 receptor was cloned in 1991 and located to chromosome 11p close to the HRAS locus (Van Tol et al., 1991; Gelernter et al., 1992). In addition to its high affinity for clozapine, this receptor is distinguished by a highly variable 48 base pair repeat in the third cytoplasmic loop, a region that is important in coupling of the receptor to G proteins (Van Tol et al., 1992). Alleles with varying lengths of the repeat from 2x48 to 10x48 base pairs have been observed. The most common repeats are the four and seven but marked variations are observed across ethnic groups (Chang et al., 1996). In all populations so far examined the four repeat is the most frequent. Alleles differ not only in length but also in the sequence and the order of the repeats (Lichter et al., 1993). In 178 unrelated chromosomes 19 different repeats in 25 different haplotypes coding for 18 different predicted amino acid sequences were identified, making this one of the most variable functional proteins currently described.

The functional significance of the exon III repeat sequence was examined by transfection experiments in COS-7 cells (Asghari et al., 1994; Asghari et al., 1995). Cloned repeat sequences were used for the reconstruction of full length cDNAs encoding D4.3, D4.5, D4.6, and D4.9 as well as the previously cloned D4.2, D4.4, and D4.7 forms. Only small differences in ligand binding were observed between the D4 repeat alleles, suggesting that the polymorphic repeat sequence has only a minor influence on G protein interaction. The functionality of the D4DR exon III repeat regions was further examined on dopamine stimulation of forskolin-stimulated cyclic AMP (cAMP) levels. The potency of dopamine to inhibit cAMP formation was about twofold reduced for D4.7 compared with the D4.2 and D4.4 variants. Again, the data presented indicate that the polymorphic repeat sequence causes only small changes in the ability of the D4 receptor to blunt cAMP
accumulation in CHO cells. Although the length of the repeat region has only small effects on ligand binding or cyclic AMP accumulation, other properties such as desensitization need to be examined before the importance or lack of importance of the exon III polymorphism is established.

Very low D4DR mRNA concentrations were detected in the striatum, whereas higher levels were observed in the prefrontal and temporo-limbic structures, brain regions involved in emotional, executive and cognitive functioning (Mulcrone and Kerwin, 1997). This pattern of gene expression correlates with the reported distribution of the D4 receptor determined by (3H) NGD 94-1 ligand binding (Primus et al., 1997). There is somewhat inconsistent evidence regarding abnormal expression of the D4DR gene in schizophrenia (Meador-Woodruff et al., 1997; Mulcrone and Kerwin, 1996; Seeman et al., 1995; Stefanis et al., 1998), but there is no evidence for either linkage or association of the D4DR receptor either to this disorder (Barr et al., 1993; Campion et al., 1994; Dollfus et al., 1996; Jonsson et al., 1996; Maier et al., 1994) or to clozapine response among patients (Kohn et al., 1997; Sanyal and Van Tol, 1997).

A null mutation in the first exon of the human dopamine D4 receptor (DRD4) gene was reported in 1994 (Nothen et al., 1994). The mutation is predicted to result in a truncated non-functional protein and was the first nonsense mutation found in a human dopamine receptor gene. It occurs with a frequency of about 2% in the general population. The distribution of the mutation was found to be similar in healthy controls compared to patients suffering from psychiatric diseases that included schizophrenia, bipolar affective disorder and Tourette's syndrome. Remarkably, an adult male was identified who is homozygous for this mutation. He showed no symptoms of major psychiatric illness, but he displayed somatic ailments including acoustic neurinoma, obesity and some disturbances of the autonomic nervous system. Some of these symptoms might be related to the absence of functional DRD4 protein.

At first, it appears quite exceptional that the subject fails to show serious behavioural abnormalities. Similarly, some knockout mice (Crabbe et al., 1996; DeVries et al., 1997; Ramboz et al., 1996; Rubinstein et al., 1997), in which various brain receptors and enzymes have been inactivated, are more or less behaviourally intact and subtle abnormalities are only revealed by use of sensitive laboratory tests. These investigations strengthen the notion that some brain receptors, and even some enzymes, may be somewhat redundant. In their absence, compensatory mechanisms come into play that apparently can ameliorate
the most serious physiological and behavioural deficits resulting from such mutations. These observations might also help explain why candidate gene and linkage studies in psychiatry and personality are often difficult to replicate.

A knockout mouse (D4R-/-) lacking the D4DR receptor has been produced (Rubinstein et al., 1997). Although less active in open field tests, D4R-/- mice outperformed wild-type mice on the rotarod and most intriguingly displayed locomotor supersensitivity to ethanol, cocaine, and methamphetamine. Biochemical analyses revealed that dopamine synthesis and its conversion to DOPAC were elevated in the dorsal striatum from D4R-/- mice. The D4DR receptor would therefore appear to modulate normal, coordinated and drug-stimulated motor behaviours as well as the activity of nigrostriatal dopamine neurons. It should be noted, however, that the D4DR receptor in rodents lacks the exon III repeat polymorphism so interesting in humans (Matsumoto et al., 1995; Ruvolo and Koh, 1996). This VNTR first appears in primates – perhaps coinciding with the development of personality traits?

THE DOPAMINE D4 RECEPTOR AND NOVELTY SEEKING

Although the evidence is strong that heritable factors account for as much as 50% of individual differences in assessment of temperament factors, only recently have specific genes been linked to particular personality traits (Benjamin et al., 1996; Cloninger et al., 1996; Ebstein et al., 1996a). In a first study of its kind, we showed that the long form of the dopamine D4 receptor (D4DR) exon III repeat polymorphism was associated with the Cloninger's TPQ Novelty Seeking trait (Figure 1). The effect size of this allele is modest and some individuals with the seven repeat score low on Novelty Seeking whereas some individuals without the allele score are high scorers on this trait. Since the D4DR long allele explains only 5% of the variance, undoubtedly several other genes are contributing to the genetic variance for this trait.

Although our finding was quickly confirmed and extended by another group of investigators using another personality questionnaire in a different population (Benjamin et al., 1996), some (Gelernter et al., 1997; Jonsson et al., 1997; Malhotra et al., 1996; Pogue-Geile et al., 1998; Sander et al., 1997b; Vandenbergh et al., 1997) but not all (Ebstein et al., 1997a; Noble et al., 1998; Ono et al., 1997) subsequent studies have failed to confirm these initial findings. It is not surprising that in some ethnic groups the effect of the D4DR is not detectable. Firstly, the contribution of this gene to Novelty Seeking is small and therefore difficult to observe. Not surprisingly, only some studies have the 'power' to detect effect sizes of <5%. Secondly, the nature of complex
traits is such that any particular gene such as the D4DR exon III repeat that only partially contributes to such traits may be replaced by alternative genes resulting in almost identical phenotypes. Any particular gene characterized by small effect size and contributing to a complex trait is neither necessary nor sufficient in the determination of the phenotype.

A third explanation, based on interactions between genes, is illustrated by several studies that simultaneously analysed the effect of common polymorphisms on personality traits. Noble (Noble, et al., 1998) and his colleagues showed that boys with the DRD4 seven repeat allele had a significantly higher Novelty Seeking score than those without this allele. However, the greatest difference in Novelty Seeking score was found when boys having all three minor DRD2 alleles and the DRD4 seven repeat allele were contrasted to those without any of these alleles. The combined DRD2 and DRD4 polymorphisms contribute more markedly to this behaviour than when these two gene polymorphisms are individually considered.

We examined in our original cohort of 120 normal volunteers two additional coding region polymorphisms, a glycine to serine substitution in the dopamine D3 receptor (D3DR) and a cysteine to serine substitution in the 5-HT2C serotonin receptor (HTR2C) (Ebstein et al., 1997b). Three-way analysis of variance (TPQ score grouped by D4DR, D3DR and 5-HT2C) demonstrated that Reward Dependence and Persistence scores were significantly reduced by the presence of the less common 5-HT2Cser polymorphism. The effect of the serine substitution in this X-linked serotonin receptor polymorphism on Reward Dependence was also observed when male and female subject groups were separately analysed. There was also a significant interaction between the two dopamine receptor polymorphisms and the serotonin polymorphism on Reward Dependence. In particular, the effect of the 5-HT2C polymorphism on Reward Dependence was markedly accentuated in individuals who had the long version of the D4DR exon III repeat polymorphism. When present in the same individual the 5-HT2C and dopamine receptor polymorphisms account for ~30% of the observed variance for Persistence (RD2) and 13% of the variance for Reward Dependence scores (RD134). The interaction between the D4DR and 5-HT2C polymorphisms in the determination of Reward Dependent behaviour has recently been confirmed in another population of middle European ancestry (W. Maier, personal communication).

A further example of interactions between common genetic polymorphisms on complex behavioural traits is illustrated by a recent study that we carried out in two-week-old neonates (Ebstein et al., 1998). Genetic effects on behaviour were evaluated at a time in early
Dopamine D4 receptor, personality and substance abuse

development when we hypothesized that environmental influences are minimal and least likely to confound associations between temperament and genes. The effect of two common polymorphisms, D4DR and 5-HTTLPR (the short s form of this promoter region polymorphism was linked by Lesch to Neuroticism or Harm Avoidance) (Lesch et al., 1996), were examined in a group of 81 two-week-old neonates who were evaluated using the Brazelton Neonatal Behavioral Assessment scale (NBAS). Multivariate tests of significance showed a significant association of D4DR across four behavioural clusters pertinent to temperament including orientation, motor organization, range of state and regulation of state (Hotelling’s T2 exact F statistic = 3.81, P = 0.007). A significant multivariate interaction was also observed between D4DR and 5-HTTLPR (Hotelling’s T2 statistic, exact F = 2.99, P = 0.02). The effect of the homozygous short 5-HTTLPR genotype (s/s) was to lower the orientation score for the group of neonates lacking the long form (L) of D4DR (Figure 2). When adult subjects were grouped by the 5-HTTLPR polymorphism there is no significant effect of L-D4DR in those subjects homozygous for the 5-HTTLPR short form (s/s) whereas in the group without the homozygous genotype the effect of L-D4DR is significant (P = 0.0006) and accounts for 13% of the variance in Novelty Seeking scores between groups. The effect of the short form of the 5-HTTLPR polymorphism (so-called Neuroticism gene) is only detectable in our studies as an interaction with a second common polymorphism, the D4DR exon III repeat region (so-called Novelty Seeking gene).

These investigations reveal the intricate relationships that exist between common polymorphisms in the determination of complex traits. Across ethnic groups expressing different allelic frequencies, the effect of any particular polymorphism on a complex trait may be either masked or amplified depending on the internal genetic milieu. For example, an abundance of long D4DR alleles in a northern European population may allow the effect of the 5-HT2C gene on Reward Dependent behaviour to be expressed, whereas in an Oriental population that exhibits a low frequency of the D4DR seven repeat (Chang, et al., 1996), the effect of the serotonin receptor on Reward Dependent behaviour may be masked.

Two recent short reviews examine the evidence that the D4DR receptor plays a role in the partial determination of Novelty Seeking (Baron, 1998; Ebstein & Belmaker, 1997).

The association of Novelty Seeking with the D4DR receptor, a gene expressed in cortical and limbic systems that regulate cognitive and emotional functions, is in accordance with Cloninger’s prediction
that Novelty Seeking is a dopaminergic trait (Cloninger, 1987). Along with Harm Avoidance and Reward, Cloninger suggested independent genetic and neurobehavioural bases for the personality factors measured by the TPQ self-report questionnaire. Harm Avoidance was predicted to be based on serotonergic mechanisms and Lesch’s recent discovery of a polymorphic region in the serotonin transporter promoter region (5-HTTLPR) that is associated with Neuroticism or Harm Avoidance (Lesch, et al., 1996) further strengthens Cloninger’s psychobiological view of temperament.

**D4DR AND SUBSTANCE ABUSE**

Our finding that Novelty Seeking was linked to the long form of the D4DR exon III repeat region polymorphism suggested that it would be worth while to examine the role of this polymorphism in substance abuse. As we discussed above, Novelty or Sensation Seeking is a personality factor that is prominent in alcoholics and heroin users. In two independent investigations, we examined D4DR exon III genotypes for a total of 198 male opioid-dependent subjects and 143 male control subjects (Kotler et al., 1997; Mel, et al., 1998). A significant increase (Figure 2) was observed in the number of opioid-dependent subjects with the seven repeat allele in comparison to the control group (28.3% vs. 14.7%; likelihood ratio = 9.2, DF = 1, P = 0.003) when D4DR genotypes were inventoried on the basis of the presence or absence of the seven allele. The relative risk for opioid dependence in individuals bearing the seven allele is 2.3 (95% CI: 1.3-4.0). Similar results were obtained if the opioid-dependent and control cohorts were inventoried by 4,4 genotype versus 4,7 genotype (28.9% vs. 16.2%; likelhood ratio = 5.39, DF = 1, P = 0.02), if all genotypes were compared (likelihood ratio = 22.9, DF = 13, P = 0.04), and if genotypes were inventoried by the long (6-8) versus short (2-5) repeat allele classification (28.9% vs. 14.3%; likelihood ratio = 7.29, DF = 1, P = 0.007).

An excess of the long form of the D4DR receptor was also observed in 121 opioid-dependent Han Chinese compared to 154 normal control subjects (Li et al., 1997). The seven allele is particularly rare in Oriental populations (Japan, Korea, and China) and only two seven repeat alleles were detected in the patient group. Overall there was an excess of longer alleles, which did not reach significance (chi 2 = 7.04; P = 0.07). When the D4DR genotypes were grouped into ‘long’ (5-7) repeats and ‘short’ (2-4) repeats, a significant excess of long alleles was observed in the patient group (P = 0.023, one-tailed), with an odds ratio of 2.30 (95% CI 1.07-4.93). These findings support the hypothesis that alleles of the DRD4 exon III VNTR are susceptibility factors for heroin abuse.
A point mutation in the aldehyde dehydrogenase 2 gene (ALDH2 allele) is considered to be a genetic deterrent for alcoholism, especially in Oriental populations where this allele occurs frequently, and the presence of this polymorphism results in unpleasant somatic sensations (flushing syndrome) when alcohol is ingested; nevertheless in a recent report, 80 of 655 Japanese alcoholics had the mutant allele (Muramatsu et al., 1996). Genotype factors that might increase susceptibility by overriding the deterrent showed a higher frequency of a five repeat allele (in Oriental populations this is a ‘long’ allele) of the dopamine D4 receptor in alcoholics with ALDH2(2) than in 100 other alcoholics and 144 controls. Alcoholics with the D4DR five repeat allele also abused other drugs more often. This investigation once again underscores the value of additional genetic information in evaluating the effects of particular alleles in contributing to complex traits. In the absence of information regarding the ALDH2(2) polymorphism, the contribution of the D4DR exon III repeat polymorphism would not be detected in this group of Japanese subjects.

As has been shown in some Caucasian populations (Benjamin et al., 1996; Cloninger et al., 1996; Ebstein et al., 1996a; 1997a; Noble et al., 1998), the D4DR exon III long alleles are also linked to Novelty Seeking, a personality trait which is perhaps a risk factor for alcoholism and substance abuse, in Japanese populations (Ono et al., 1997). The Novelty Seeking subscale of Exploratory Excitability had a significant association with long alleles of the polymorphic exon III repeat sequence of D4DR in a group of 153 Japanese nursing students.

Although these studies have linked the D4DR long allelic forms to substance abuse in some populations, a number of other studies have failed to find an association between D4DR and alcoholism (Adamson et al., 1995; Chang et al., 1997; Geiger et al., 1997; Malhotra, et al., 1996; Parsian et al., 1997; Sander et al., 1997a; Sullivan et al., 1998). We have discussed above some reasons for the failure to confirm initial associations such as that between the D4DR exon III repeat length polymorphism and substance abuse as well as the antecedent personality trait of Novelty Seeking.

Although the association between D4DR and Novelty Seeking assessed by self-report questionnaires has been weakened by subsequent studies, a number of investigations have reported a role of this polymorphism in behaviours (in addition to substance abuse discussed above) characterized by Novelty and Sensation Seeking including attention deficit hyperactivity disorder (LaHoste et al., 1996; Swanson et al., 1998) and compulsive gambling (Perez de Castro et al., 1997). In particular, the accumulating evidence that ADHD children (LaHoste,
et al., 1996; Swanson, et al., 1998), often characterized as impulsive and disruptive, are characterized by an excess of the D4DR long alleles strengthens the notion that this gene also contributes to Novelty or Sensation Seeking behaviours in adults.

SUMMING UP
The road from genes to substance abuse follows a non-linear pathway modulated by moderately heritable temperament traits. Genes do not directly code for ‘substance abuse’; there are only complex interactions by combinations of genes of small effect size that subtly influence temperament and in combination with powerful environmental forces create a personality profile that is susceptible to this kind of maladaptive behaviour. In particular, there is some evidence that the long alleles of the dopamine D4 exon III receptor contribute in some populations to Novelty or Sensation Seeking behaviour that in turn is a risk factor for substance abuse. Differences in allele frequency between ethnic groups and interactions between common polymorphisms may explain failure to replicate initial findings that seem to demonstrate associations between candidate genes and complex traits. We suggest that as more genetic information regarding common polymorphisms becomes available in substance abusers defined across various ethnic and population divisions, the reasons for apparent failures to validate early findings may be resolved and explained by interactions between common alleles.

TABLE 1 (opposite)
TPQ scores compared between opioid-dependent subjects and non-addicted control subjects. Subjects were Jewish males, non-Ashkenazi mainly of North African origin. REW134 is reward-dependent behaviour (Mel et al., 1998).
## TPQ Scores for Addicts and Matched Controls

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FIGURE 1
TPQ Novelty Seeking scores grouped by the presence or absence of the long, 7 repeat allele of the D4DR receptor (Ebstein et al., 1996b). The mean Novelty Seeking Score for the group with the 7 repeat allele was significantly higher than the group without the 7 repeat (P = 0.01). The effect size (eta squared) was 0.05.
FIGURE 2
Brazelton Neonatal Behavioural Assessment Scale (NBAS) Orientation scores in a group of neonates grouped by the D4DR and 5-HTTLPR polymorphisms (Ebstein et al., 1998).
FIGURE 3
Allele frequency (%) of D4DR exon III repeat polymorphism in opiate-dependent and control subjects (Kotler, et al., 1997; Mel, et al., 1998).
REFERENCES


Genes, behaviour and health
THE DOPAMINE HYPOTHESIS OF SCHIZOPHRENIA: NEW INSIGHT FROM BRAIN IMAGING STUDIES

Marc Laruelle

ABSTRACT
The dopamine hypothesis of schizophrenia proposes that hyperactivity of dopaminergic transmission is associated with this illness, but direct observation of abnormalities of dopamine function in schizophrenia has remained elusive. We used a newly developed single photon emission computerized tomography (SPECT) method to measure amphetamine-induced dopamine release in the striatum of thirty patients with schizophrenia and fifteen healthy controls. Amphetamine-induced dopamine release was estimated by the amphetamine-induced reduction in dopamine D2 receptor availability, measured as the binding potential of the specific D2 receptor radiotracer $[^{123}]$I-$(-)$-3-ido-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinylmethyl] benzamide ($[^{123}]$IIBZM). The amphetamine-induced decrease in $[^{123}]$IIBZM binding potential was significantly greater in the schizophrenic group (Mean ±SD, -17±13%) compared to the control group (7±7%). In the schizophrenic group, elevated amphetamine effect on $[^{123}]$IIBZM binding potential was associated with emergence or worsening of positive psychotic symptoms. This result suggests that psychotic symptoms elicited in this experimental setting in schizophrenic patients are associated with exaggerated stimulation of dopaminergic transmission. Such an observation is compatible with an abnormal responsiveness of dopaminergic neurons in schizophrenia. These results illustrate how new brain imaging techniques enable noninvasive study of specific synaptic activities in the living human brain, and elucidation of neurochemical dysfunctions associated with severe mental illness.

The “classical” dopaminergic hypothesis of schizophrenia proposed that hyperactivity of dopamine (DA) transmission is responsible for at least some symptoms of the disorder. This hypothesis is supported by the correlation between the antipsychotic potency of neuroleptics and their potency to block DA D2 receptors, and by the psychotogenic
effect of amphetamine and other DA enhancing drugs. Since positive symptoms (i.e. hallucinations and delusions) are more sensitive than negative symptoms (withdrawal, lack of drive, alogia) to direct manipulation of the DA system, hyperactivity of DA transmission is likely to be more relevant to positive than negative symptoms. These pharmacological effects suggest, but do not establish, a dysregulation of DA systems in schizophrenia.

Despite decades of effort to validate this hypothesis, documentation of abnormalities of DA function in schizophrenia has remained elusive. Postmortem studies measuring DA and its metabolites in the brain of schizophrenic patients have yielded inconsistent results. Increased density of striatal D2 and D2-like receptors, reported in most but not all postmortem studies, has been difficult to interpret, given that neuroleptic drugs upregulate these receptors. PET and SPECT studies of striatal D2 and D2-like receptors density in neuroleptic-naive schizophrenic patients have been inconclusive: while one group reported increased striatal D2-like receptor density in schizophrenia, other groups reported negative results. The lack of clear evidence for increased dopaminergic indices in schizophrenia might indicate that DA transmission is enhanced only relatively to other systems, such the glutamatergic systems. On the other hand, the absence of data supporting the DA hypothesis of schizophrenia might be due to the difficulty of obtaining direct measurement of DA transmission in the living human brain.

This situation is rapidly changing. Several groups have recently provided evidence that competition between neurotransmitters and radioligands for neuroreceptor binding allows measuring changes in synaptic neurotransmitter levels with in vivo binding techniques. In rodents, decreased uptake of D2 radioligands has been measured following administration of amphetamine and other DA-enhancing drugs, whereas the opposite effect (i.e. increased tracer accumulation) has been induced by drugs which decrease DA concentration. In baboons, decreased specific uptake of PET or SPECT D2 radiotracers has been reported following amphetamine challenge. In humans, decreased accumulation of the D2 antagonists has been observed following challenges with amphetamine or methylphenidate. Thus, reduction in D2 receptors availability, measured as the displacement of radioligand, might provides a noninvasive measure of DA release. We used this imaging technique to study DA transmission following acute amphetamine challenge in schizophrenia. In this paper, we will review the main steps of the development of this technique, as well as its application to schizophrenia research.
STUDIES IN BABOONS
We initially observed with SPECT that the striatal washout rate of the specific D2/D3 receptor radiotracer [123I]iodobenzamide ([123I]IBZM) in baboons was significantly increased by amphetamine. Since amphetamine has negligible affinity for [123I]IBZM binding (in vitro IC50 of amphetamine for [125I]IBZM binding > 10 ÅM, unpublished results), this effect could not be attributed to a direct displacement of [123I]IBZM by amphetamine. [123I]IBZM is a highly lipophilic compound that binds with only moderate affinity to D2 receptors, both properties suspected to enhance vulnerability to competition by endogenous DA. Since amphetamine is a potent DA releaser, we postulated that the increased [123I]IBZM washout reflected a reduction in D2 receptors availability due to increased DA synaptic concentration and increased D2 receptor occupancy by DA. Yet, the washout rate of a tracer after single bolus injection is also influenced by blood flow, which is affected by amphetamine. Because of this difficulty, we investigated the amphetamine effect under sustained equilibrium conditions during tracer constant infusion experiments.

[123I]IBZM was administered as a priming bolus followed by a continuous infusion at a constant rate for the duration of the experiment. This method of administration induces a state of sustained binding equilibrium which provides a stable baseline to evaluate changes in receptor availability (Fig. 1A). Under these conditions, we observed that amphetamine decreased the striatal specific binding (Fig. 1B). The binding potential (BP), the parameter measuring receptor availability, is equal to the product of the receptor density (Bmax) and affinity (1/KD). We have previously demonstrated, both theoretically and experimentally, that, under sustained equilibrium conditions achieved by radiotracer constant infusion, the decrease in specific to nonspecific equilibrium ratio (denoted V3") is equal to the decrease in BP. Therefore, under constant infusion conditions, the amphetamine-induced decrease in D2 receptor BP can be readily calculated as (Sa-Oa)/(Sa-Ob)/Ob where S is striatal total activity, O is occipital activity (a region with negligible number of D2 receptors), the subscripts "a" and "b" indicate measures after and before amphetamine, respectively. Because measurements are obtained at equilibrium, these ratios are independent of amphetamine effects on cerebral blood flow and on peripheral clearance. Thus, the reduction in [123I]IBZM-specific binding observed after amphetamine reflects a decrease in D2 receptor BP, and not an effect of amphetamine on blood flow and peripheral clearance.

To establish that the amphetamine-induced reduction in [123I]IBZM BP is mediated by DA release, we repeated these experi-
ments following alpha-methyl-para-tyrosine (AMPT) pretreatment. AMPT is a competitive inhibitor of tyrosine hydroxylase, the rate limiting enzyme for DA synthesis. Using microdialysis, we observed an 83% reduction in amphetamine-induced DA release measured following acute AMPT pretreatment (n = 3). Following the same dose of AMPT, the amphetamine effect on $[123I]$IBZM BP was attenuated by an average of 66% of control values (n = 2). These experiments confirmed that the reduction in $[123I]$IBZM BP following amphetamine is mediated by DA release and a higher D2 receptor occupancy by DA.

Since we developed this method as a potential noninvasive measure of DA release, it was crucial to define the relationship between the magnitude of DA release and the reduction in $[123I]$IBZM BP. We observed that, in baboons, the reduction in $[123I]$IBZM BP measured with SPECT following various doses of amphetamine was linearly correlated with the peak DA release measured with microdialysis (Fig. 2). This observation validated the use of this noninvasive paradigm to measure the increase in DA synaptic concentration after amphetamine challenge. We also evaluated the reproducibility of the SPECT measurement of the amphetamine effect on D2 BP by repeating three experiments at 0.3, 0.5 and 1 mg/kg, respectively. The reproducibility of the amphetamine effect was excellent. The average difference between test and retest in DBP was 2 ± 2% (n = 3, with these and subsequent values given Mean ± SD), which corresponded to an intraclass correlation coefficient of 0.97. In conclusion, these experiments in primates established that the reduction in $[123I]$IBZM BP measured with SPECT following amphetamine challenge can be used as a noninvasive measurement of the magnitude of the amphetamine-induced increase in synaptic DA.

**STUDIES IN HEALTHY HUMAN VOLUNTEERS**

Eight young healthy volunteers (age 26 ± 3 years) were first studied with $[123I]$IBZM bolus plus constant infusion protocol under control conditions (i.e. no amphetamine injection). Under this protocol, stable levels of striatal and background (occipital) activities were maintained from 150 min to the end of the experiment. The experiment was repeated under similar experimental conditions, except that 0.3 mg/kg amphetamine was injected i.v. at 240 min. Amphetamine 0.3 mg/kg i.v. induced a significant decrease in $[123I]$IBZM BP. Behavioural effects of the amphetamine injection were rated by the subjects with a simplified version of the Amphetamine Interview Rating Scale. We observed a significant correlation between the euphoria, alertness and restlessness, and the reduction in $[123I]$IBZM BP ($r^2 = 0.84, P = 0.003$), confirming that DA mediates these emotions after amphetamine. We
also tested the reproducibility of the amphetamine effect in healthy subjects: four subjects underwent repeated experiments at one-week intervals. Amphetamine-induced reduction in [123I]IBZM BP had an excellent intraclass correlation coefficient (0.80). No tolerance or sensitization to amphetamine effect on DA release was observed in these experiments.

**STUDIES IN PATIENTS WITH SCHIZOPHRENIA**

Sustained, but not acute, use of amphetamine in humans is well known to produce a psychotic state characterized by some salient features of paranoid schizophrenia. Patients with schizophrenia are more vulnerable than healthy controls to the psychotogenic effects of acute administration of amphetamine. Meta-analysis of the available literature in 1987 revealed that approximately 40% of patients with schizophrenia worsen with psychotic symptoms after an acute psychostimulant challenge (versus 2% in non-schizophrenic psychiatric patients and 0% in healthy controls) while 20% improved and 40% were unchanged. Thus, patients with schizophrenia are vulnerable to acute challenge with amphetamine at doses that are not psychotogenic in healthy individuals. We used the technique described above to study the pathophysiology of this abnormal behavioural response to amphetamine in schizophrenia.

No difference was observed in the baseline (i.e. pre-amphetamine) [123I]IBZM BP between patients (mean±SEM, 224±92 mL g-1) and controls (221±63 mL g-1), in accordance with previously published results. In contrast, the amphetamine-induced decrease in [123I]IBZM BP was significantly larger in schizophrenic patients (-17±13 %) than in controls (-7±7%; P = 0.001, Fig. 3). This group difference could not be attributed to differences in amphetamine disposition, since amphetamine plasma levels were similar in patients (29±10 ng/mL) and controls (28±11 ng/mL). In the schizophrenic group, no correlation was observed between the amphetamine response and the duration of the neuroleptic-free interval or the lifetime exposure to neuroleptic medications.

Amphetamine-induced changes in positive psychotic symptoms were measured with the Positive and Negative Symptom Scale (PANSS). We defined a clinically significant psychotic reaction to amphetamine as a 4 points or greater increase over baseline of the positive symptoms subscale of the PANSS. Amphetamine induced a transient but clinically significant worsening in positive psychotic symptoms in 12 out of 30 patients (40%). This distribution was consistent with the previously reported prevalence of psychotic reactions to acute challenges with DA agonists in schizophrenia. Psychotic reactions were characterized mostly
by delusional paranoid ideations ("The CIA is watching me because I am responsible for the bombing of the US embassy in Lebanon") and hallucinations ("The angels are here, I can see them") that were not present at baseline. Amphetamine-induced psychotic reactions were transient and, in all cases, patients recovered their baseline state a few hours after the challenge. No psychotic symptoms were observed in healthy controls. Schizophrenic patients who experienced worsening in positive symptoms showed larger reductions in [123I]IBZM BP (mean±SEM, -25±4%, n = 12) than schizophrenic patients whose positive symptoms did not worsen (-11±3%, n = 18) and healthy controls (-7±1%, n = 30, ANOVA: p < 0.001). In the schizophrenic group, the magnitude of the amphetamine effect on [123I]IBZM BP was positively correlated with changes in positive symptoms (r = 0.53, P = 0.001, Fig. 4).

DISCUSSION
This study represents the first attempt to measure in vivo striatal DA release in patients with schizophrenia. The data indicate that more D2 receptors are occupied by DA following amphetamine challenge in schizophrenic patients than in matched healthy controls. These results have been independently replicated with PET, [11C]raclopride and a lower dose of amphetamine (0.2 mg/kg). These studies suggest a state of dysregulation of presynaptic DA activity in schizophrenia, a conclusion that would be consistent with two recent PET studies showing increased accumulation of the DA precursor 6-[18F]fluoro-L-dopa in the striatum of patients with schizophrenia (Reith et al., 1994; Heitala et al., 1995).

The increased displacement of [123I]IBZM binding following DA release observed in the schizophrenic group could reflect either an increased affinity of D2 receptors for DA or an increased concentration of DA in the vicinity of the receptors, or some combination of both factors. Available data do not support the existence of an increased affinity of D2 receptors for agonists in schizophrenia: the sequence of the D2 receptor gene is not altered and the binding of DA agonists in postmortem striata is not increased in schizophrenia. Nevertheless, a decreased DA concentration at baseline would result in an effective increased affinity of the unoccupied D2 receptors (for both agonists and antagonists). Again, available data do not support the existence of a marked reduction in baseline DA in schizophrenia, since the in vivo affinity of [11C]raclopride is not elevated in patients with this condition. Therefore, while a contribution of the affinity factor can not be definitively excluded, an increased concentration of DA in the vicinity of the
receptors is likely to be the predominant mechanism underlying the observed effect.

The mechanism of this putative increased dopaminergic neuronal reactivity remains to be elucidated. Corticofugal glutamatergic projections that increase the responsiveness of dopaminergic subcortical systems are inhibited by dopaminergic prefrontal projections, both directly and indirectly via GABAergic interneurons. This glutamatergic cortical control occurs primarily through projections to the DA cell body area rather than the terminal region. In nonhuman primates, selective destruction of DA terminals in dorsolateral, medial and orbital regions of the prefrontal cortex does not affect striatal baseline DA concentration but induces a long-lasting increase in striatal potassium-induced DA release. Since potassium, like amphetamine, stimulates both DA synthesis and release, this observation is potentially relevant to the present findings. Thus, the increased responsiveness of subcortical DA neurons observed in this study might be secondary to prefrontal dopaminergic or GABAergic deficits as both deficits have been proposed as constituents of the “cortical pathology” in schizophrenia (Weinberger, 1987; Benes et al., 1991).

In conclusion, this study used a newly developed noninvasive method to measure amphetamine-induced DA release in patients with schizophrenia and suggested the existence of a dysregulation of DA neurons in schizophrenia leading to an increased DA transmission in response to amphetamine. This observation provides direct support for the time-honoured dopaminergic hypothesis of schizophrenia.

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FIGURE 1
A. Striatal (closed circles) and occipital (open circles) activities during bolus plus constant infusion with $[^{123}]$IBZM. The constant infusion created a state of sustained equilibrium (both striatal and occipital activities were stable over time). B. The experiment was repeated in the same baboon, under similar experimental conditions, except for the injection of 0.3 mg/kg amphetamine at 240 min. This injection induced a decrease in striatal activity (specific activity) but did not affect the occipital activity (nonspecific activity).
FIGURE 2
Correlation between amphetamine-induced peak DA release, measured with microdialysis (y axis) and the decrease in [123I]IBZM D2 BP, measured with SPECT (each point is the mean of three experiments).
FIGURE 3
Amphetamine-induced relative decrease in [123I]IBZM binding potential in 30 healthy controls and 30 patients with schizophrenia, matched for age, sex, race and parental socioeconomic level.
FIGURE 4
Relationship between amphetamine-induced changes in positive symptoms and amphetamine-induced relative decrease in [123I]IBZM binding potential in the schizophrenic group.
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Genes, behaviour and health
LIFE-LONG PLASTICITY OF THE NEUROBEHAVIOURAL PHENOTYPES: THE DIATHESIS-STRESS HYPOTHESIS OF PSYCHOPATHOGENESIS

S. Puglisi-Allegra and S. Cabib

INTRODUCTION
Phenotype is defined as any observed characteristic of the individual, ranging from cell structure to locomotion pattern. Genotype refers to the inferred hereditary elements that make the phenotype of one individual different from the phenotype of another individual. All phenotypic characteristics, including behaviour, have a genetic correlate or origin but this does not mean that they are determined by genes. Instead, phenotypes are the results of the developmental interactions of the organism and its environment. The idea that phenotypes are a developmental outcome may be misleading in the sense that it could suggest that genetic factors and environment contribute to the development of a phenotype only in immature organisms or in some critical early stage of life, remaining fixed thereafter. Instead, development is a life-long phenomenon defined by the ability of the organism to reorganize and change in the face of a changing environment (Schneirla, 1966). Moreover, longitudinal genetic analyses during childhood do not completely overlap from age to age, indicating changes in genetic effects (Plomin et al., 1994). These considerations imply life-long plasticity of phenotypes and this is not surprising. Hair colour, body size and behaviour may markedly change with age and these changes are susceptible to environmental modulation.

Psychopathological changes involve behaviour and neural functioning; thus they contribute to the development of what might be called the neurobehavioural phenotype of an individual. The last decades have seen major developments in genetic analysis of behavioural disorders. A wealth of data derived from family, twin and adoption studies support the view of a major role of genetic liability for schizophrenia and affective disorders, including major depression and manic-depressive

On the other hand, although the current interest in molecular biology has produced a strong focus on testing a single-gene model of genetic transmission (Mullen & Murray, 1989; Pardes et al, 1989), attempts to fit data within a major-gene model have generally met with failure (Faraone and Tsuang, 1985; Rish, 1990; Gottesman & McGue, 1991). These failures may reveal the limitation of this approach for studying genetic influence on behaviour that, as repeatedly pointed out, involves multiple genes, rather than one or two major genes, as well as non-genetic sources of variance (Plomin, 1990).

As for non-genetic, environmental contributions to psychopathology, increasing attention has been devoted to the involvement of stressful experiences (Bidzinska, 1984; Gottesman & Shields 1982; Willer, 1991; Fowels, 1992, for a review). The list of pathogenic life events includes experiences over the whole life cycle. Some, such as marriage (Holmes & Rahe, 1967), may only involve adults. This supports the hypothesis of life-long plasticity of neurobehavioural phenotypes. The most relevant conceptualization of stress involvement in psychopathogenesis is the diathesis-stress hypothesis proposed for the etiology of schizophrenia (Rosenthal, 1970; Fowels, 1992). According to this hypothesis, environmental factors (stress) promote pathological outcomes in the presence of a genetic liability and, in addition, they are not specific for a given pathology whereas the genetic factors (diathesis) are.

These considerations entail a major role for the contributions from animal studies in preclinical research. Indeed, animal research allows manipulations of genetic as well as non-genetic variables and detailed studies on cellular and subcellular components of the central nervous system that may play a crucial role in the development of “weak” points underlying susceptibility or vulnerability. In the following pages we review the contribution offered by such research.

GENOTYPE-ENVIRONMENT INTERACTIONS
IN THE DEVELOPMENT OF INDIVIDUAL LIABILITY TO
PSYCHOPATHOLOGY

The diathesis-stress hypothesis (Rosenthal, 1970; Fowels; 1992) indicates two dimensions for environment-gene interaction in the development of psychopathology. The first is liability: it suggests cumulative effects of environmental pressure (stress, life events) and genetic fragility in determining individual proneness to developing pathological outcomes. Thus, for extreme genetic fragility, contribution from environmental sources would be nearly irrelevant (i.e. x-fragile and other
genetic pathologies). However, extreme environmental pressure may promote pathology in genetically resistant individuals (post-traumatic stress disorders, syndromal depression). Between these two extremes lies a large segment of cases in which the contribution of moderately severe experiences is both necessary and sufficient to promote the expression of pathology in genetically fragile individuals.

It should be pointed out that both gene and previous stress experiences may modulate individual sensitivity to the stressors by determining individual ability to cope behaviourally with environmental changes. Exploration of an unfamiliar environment is a routinely-used test for emotional reactivity in rodents. Animals are introduced into open, usually brightly lighted arenas, which are not only novel but also aversive for a nocturnal species which inhabits burrows. Thus, this condition may represent a stressful experience for the animals, which explore the environment to find a way out or a safe hide (Misslin et al., 1982). The amount as well as the pattern of exploration exhibited by rodents in these conditions is modulated by the degree of aversiveness (lighting, dimension of the open space, presence or absence of hiding places) and by the emotional reactivity of the subjects.

Highly emotional animals are less explorative, spend more time in the more protected areas and tend to freeze. Several inbred strains have been tested in these conditions, revealing large strain-dependent differences for the amount as well as the pattern of exploration. An inbred strain is a set of animals that is produced by at least 20 consecutive generations of sister x brother or parent x offspring mating and that can be traced to a single ancestral pair in the 20th or subsequent generations. Animals of an inbred strain are almost fully homozygous, which provides a well-defined and consistent genotype for analysis (Oliverio et al., 1992). However, these animals are also reared in virtually identical environments. In an outbred population (a genetically variable population) exploration of unfamiliar environment has been demonstrated to depend on perinatal experiences (Vallé et al., 1997). Thus, the offspring of dams subjected to stressful experiences during pregnancy are less active in a novel environment than the offspring of the unhandled controls. Instead, manipulation of pups at the early stage of postnatal life produces highly explorative and low emotional adults (Cabib et al., 1993). Interestingly, recent results indicate that the effects of early manipulation are mediated by its indirect effects on maternal behaviour (D'Amato et al., 1998).

Finally, experimental studies in laboratory animals have also demonstrated the role of interactions between genetic and experiential factors in the development of emotional reactivity to aversive experiences. DeFries and co-workers (1967) observed that daily physical stress throughout
the latter half of pregnancy in two strains of mice produced offsprings whose level of activity in an open field was a function of both fetal and maternal genotype. That is, mothers of one strain responded to the physical stress differently from the mothers of the other strain, and their reactions produced a uterine environment which, interacting with fetal genotype, promoted the development of individuals that differed in exploratory tendencies and fearfulness in adult life.

Moreover, both genes and experiences may contribute to produce stressful conditions, thus increasing liability derived from environmental sources. Indeed, behavioural and cognitive disabilities interfering with social interactions or personal achievement may promote negative attribution to events. Mice previously defeated by an aggressive male conspecific show species-specific patterns of defensive/submissive behaviour during subsequent social interactions. Since submissive behaviour tends to elicit aggressive-dominant reaction by the interacting male the altered pattern of social behaviour may increase chances of stressful social experiences (see Puglisi-Allegra et al, 1989, for review). On the other hand, it has been shown that previous exposure to unescapable shock impairs subsequent learning of avoidance behaviour (Maier et al, 1969), indicating that previous experiences with an unavoidable stressor may have disruptive effects on coping responses, leaving the organism helpless in the face of environmental threat. Interestingly, comparison of these effects of unavoidable shock in inbred strains of mice revealed dramatic differences further supporting the major role of the interaction between genetic factors and environmental pressure (Zacharko & Anisman, 1991).

**GENOTYPE-ENVIRONMENT INTERACTIONS IN THE DEVELOPMENT OF SPECIFIC PSYCHOPATHOLOGICAL OUTCOMES.**

The second dimension for gene-environment interaction proposed by the diathesis-stress hypothesis of psychopathology concerns the characteristics of the phenotype that will develop. The diathesis-stress hypothesis states that the genotype determines the typology of pathological outcomes promoted by stress. However, the cross-talk between gene and the environment in which the organism lives is not direct. Genes interact with other entities inside a cell and organisms interact with the outside world that is their environment. Moreover, any organism, whatever the phase of its life-long development, has a history.

An increasing amount of evidence points to alterations of dopamine (DA) receptors in different types of psychopathologies (Seeman et al, 1993; Schmauss et al, 1993; Pearson et al, 1995; Murray et al, 1995).
Nevertheless, linkage studies do not support a role of genes encoding for the different types of DA receptor in such pathologies (Wiese et al., 1993; Bjerley et al., 1994; Campion et al., 1994; De Bruyn et al., 1994; Macciardi et al., 1994; Nanko et al., 1994). These discrepancies suggest that specific alterations of brain DA receptors may arise from the impact of stress on genetic susceptibility involving other factors besides genes for DA receptors.

Such a possibility was recently tested in laboratory animals (Cabib et al., 1997). Following repeated or chronic stressful experiences, mice of the C57BL/6 and DBA/2 strains show distinct, strain-dependent behavioural disturbances. Stressed DBA/2 mice show enhanced locomotor response to amphetamine challenge (Badani et al., 1992; Cabib et al., 1995; Cabib & Bonaventura, 1997), reduced sensitivity to behavioural inhibition promoted by acute stress experiences (Puglisi-Allegra et al., 1990; Cabib et al., 1995; Cabib & Puglisi-Allegra, 1996), and spontaneous stereotypes (Cabib & Bonaventura, 1997). Instead, stressed mice of the C57BL/6 strain show changes (Cabib & Bonaventura, 1997) or reduced (Badani et al., 1992) locomotor response to amphetamine, enhanced sensitivity to behavioural inhibition promoted by acute stress experiences (Puglisi-Allegra et al., 1990; Cabib & Puglisi-Allegra, 1996) and no sign of spontaneous stereotypes (Cabib & Bonaventura, 1997).

The behavioural responses affected in a strain-dependent manner by stressful experiences are all considered preclinical models of psychopathologies (Segal & Schuckit, 1983; Robinson, 1988; Lyon, 1991; Willer, 1991; Robinson & Berridge, 1993; Cabib & Puglisi-Allegra, 1996). Moreover, these behavioural effects are related to brain DA functioning (Puglisi-Allegra et al., 1990; Badani et al., 1992; Cabib et al., 1995; Cabib & Bonaventura, 1997; Cabib & Puglisi-Allegra, 1996).

Finally, converging evidence points to mesoaccumbens DA receptors as the neural substrate of the strain-dependent effects of stress. Indeed, some of the major effects of stress involve D2-like receptors in the mesoaccumbens DA system which also show the most relevant strain-dependent differences in unstressed mice (Cabib et al., 1998). Moreover, the stress effects were opposite to the initial strain differences. In comparison with C57BL/6, DBA/2 mice are characterized by a higher density of D2-like receptors in the ventral tegmental area (VTA). The same stressful condition reduces VTA DA receptors in DBA/2 mice and increases them in C57BL/6 mice (Cabib et al., 1998).

The latter finding, indicating that stress promotes opposite strain-dependent alterations of mesoaccumbens DA autoreceptor densities, fits with previous results which demonstrated opposite
stress-induced alteration of mesoaccumbens DA autoreceptor sensitivity in the two strains (Cabib & Puglisi-Allegra, 1991). Strain-dependent differences are not sufficient to establish the role of genetic factors in the control of phenotypes. However, a classic genetic analysis as well as an analysis of quantitative trait loci (QTL) in recombinant inbred strains have been conducted on a behavioural index of DA autoreceptor sensitivity in stressed mice (Cabib et al, 1985; 1997). These results indicate that mesoaccumbens DA autoreceptor density is a polygenic trait controlled by a major genotype x stress interaction. This finding is relevant to clinical research since it supports the view that different pathological profiles may derive from genotype-dependent adaptation of brain systems to environmental pressure and points towards an involvement of brain DA autoreceptors in this adaptation process.

Finally, the results obtained by the QTL analysis strongly suggest an involvement of regulatory factors related to stress response (such as CRH and steroid-related products) and neural or synaptic plasticity (such as rate-limiting factors for protein synthesis, potassium channel proteins, glutamate and gangliosides) in the control of brain DA receptor plasticity under environmental pressure (Cabib et al, 1997). This animal model describes the diathesis-stress condition in which specific alterations of brain DA receptors arise from the impact of stress on genetic susceptibility involving other factors besides genes for DA receptors suggested by clinical research.

Drugs of abuse may be considered as pathogenic agents which, by interacting with susceptible phenotypes, promote a pathological behaviour characterized by compulsive drug seeking (addiction). A number of animal studies demonstrate a major role of genotype on the one hand (Crabbe et al, 1994) and of stressful experiences on the other (Piazza & Le Moal, 1996) in the development of a susceptible phenotype. These studies have also identified two inbred strains (C57BL/6 and DBA/2) as models for genetically-related susceptibility to drug abuse and a number of behavioural and neural parameters which characterize individuals with high propensity to drug consumption (Piazza & Le Moal, 1996). It has been suggested that the strong relationship between these parameters in addiction-prone individuals is due to the fact that they are all indices of the activity of brain systems mediating positive motivation. Indeed, the intensity of positively reinforced behaviour or goal-directed behaviour is thought to depend on the activity of dopaminergic neurons projecting towards the nucleus accumbens. Thus, individuals susceptible to drug abuse could be characterized
by a facilitated responsivity of these neurons, possibly due to corticoïd-dependent factors (Piazza & Le Moal, 1996).

Interestingly, the differences between the two inbred strains of mice for these parameters are those expected on the basis of their relative susceptibility to drug-taking. When compared with the low-responding DBA/2 mice, high-responding C57BL/6 mice show higher locomotor response to a novel environment (Puglisi-Allegro & Cabib, 1997), more prolonged increase in plasma levels of corticosterone (Cabib et al, 1996), and enhanced locomotor response to psychostimulants related to enhanced release of the neurotransmitter dopamine from terminals located in the nucleus accumbens septi (Zocchi et al, 1998). However, recent experiments indicate that a chronic stressful experience (food restriction) produces dramatic changes in several of the parameters related to drug abuse susceptibility in mice of the DBA/2 strain. Thus, food-restricted DBA/2 mice show enhanced locomotor response to novelty and to amphetamine challenge (Cabib & Bonaventura, 1997) as well as increased sensitivity towards the positive reinforcing effects of the psychostimulant (Figure 1). As for the locomotor-activating effects of amphetamine, stressed DBA/2 mice appear to be even more responsive than control or stressed mice of the C57BL/6 strain (Cabib & Bonaventura, 1997). Finally, the enhanced responsivity to the psychomotor effects of amphetamine in food-restricted DBA/2 mice persists over several days of subsequent free feeding (Cabib & Bonaventura, 1997). These data suggest that environmental pressure can change neurobehavioural phenotypes related to susceptibility to drug addiction in the mature organism.

The absence of stress-induced changes for the psychomotor effects of amphetamine in mice of the C57BL/6 strain suggests low, possibly genotype-dependent, sensitivity to environmental pressure. However, opposite conclusions may be reached from the evaluation of different behavioural phenotypes and different stressful conditions.

Despair and helplessness are considered to be model symptoms of depression in laboratory animals as well as the inability to initiate or sustain active defensive strategies (escape, avoidance) in aversive conditions. These behavioural disturbances are promoted by previous exposure to uncontrollable/unavoidable stress experiences (see Cabib & Puglisi-Allegro, 1996, for review). One experimental paradigm routinely used in rodents is the so-called forced swimming test (FST). Animals are individually placed in a cylinder containing warm water in a quantity that allows the animal to float and swim. The initial response by the subject is characterized by attempts to escape the situation which, however, is devoid of ways out. With the passage
of time, the animals progressively reduce escape attempts and assume a state of rigid immobility. Upon a second exposure to the situation, animals make little attempt to escape and show high levels of immobility that is considered to model a helpless behavioural response to an uncontrollable/unavoidable aversive condition. Pharmacological studies have shown that immobility in the FST is reduced by clinically effective antidepressants, suggesting a good predictive validity of this animal model of depression (Cabib & Puglisi-Allegra, 1996). When tested for FST-induced immobility, mice of the DBA/2 strain show the classical time-dependent increase; instead, mice of the C57BL/6 strain immediately show high levels of immobility. The re-exposure to the test conditions increases immobility in DBA/2 mice to the levels initially shown by C57BL/6 mice. The latter show a further dramatic increase in this response. These data suggest that C57BL/6 are more rather than less susceptible to unavoidable/uncontrollable stress than DBA/2 mice (Figure 2).

Unavoidable/uncontrollable stressors inhibit mesoaccumbens dopamine release (Puglisi-Allegra et al, 1990; 1991; Cabib & Puglisi-Allegra, 1994). It has been suggested that this response may be responsible for emotional-motivational withdrawal from aversive events for which no coping behaviour is found. When monitored during exposure to the FST, dopamine release in the nucleus accumbens of C57BL/6 mice shows an almost immediate decrease, whilst in DBA/2 mice an initial increase of neurotransmitter release is followed by a decrease (Figure 2). The parallelism between behavioural and mesoaccumbens DA response to the FST supports the view that there is a strong relationship between behavioural despair and inhibition of mesoaccumbens DA. It strengthens the hypothesis that C57BL/6 mice are more susceptible to unavoidable/uncontrollable stressors and suggests that some qualitative characteristic of the stress experience may indeed be relevant for determining stress outcomes.

CONCLUSIONS
In the present review we have considered the results obtained by pre-clinical research in laboratory animals in the context of the diathesis-stress hypothesis of psychopathology. These results demonstrate complex interactions between genotype and stress in determining behavioural and neural disturbances within a model proposed to study psychopathology in laboratory animals. The behavioural and neural changes promoted by the interaction between genotype and stress in adult animals are consistent and support the view that neurobehavioural phenotypes are highly plastic in mature organisms.
The term "interaction" applied to the relationship between genotype and stress may be misleading since stress here is intended as a psychological experience by the organism in the face of more or less dramatic environmental changes. Genes do not come into direct contact with the external environment, i.e. with the world outside the organism's skin. However, they interact with other entities inside the cell which can be influenced by the outside world, as in the case of stress effects on brain DA receptors. On the other hand, as discussed in the case of individual liability, organisms determine some relevant aspects of their environment through behaviour which is itself influenced by genes.

Thus, the diathesis-stress hypothesis of psychopathology may help in understanding how these different and distant levels of the organism-environment organization may participate in the life-long development of neurobehavioural phenotypes.
FIGURE 1
Behavioural effects of amphetamine in two inbred strains of mice (upper panel) and in chronically stressed mice of the DBA/2 strain (lower panel). Place preference scores (P/T) indicate preference for the side of a test cage previously paired with amphetamine effects. Mean crossings indicate locomotor response to amphetamine. Mice of the C57BL/6 strain show preference for the drug paired side of the test cage at all doses tested whilst mice of the DBA/2 strain show avoidance or no choice. However, a chronic stressful experience changes the avoidance for the drug paired cues into preference in DBA/2 mice. As for the locomotor effects of the psychostimulant, they are lower in DBA/2 mice in comparison with C57BL/6 but, following stress, the latter show a response to a dose of amphetamine that is ineffective in unstressed C57BL/6 mice.
FIGURE 2
Behavioural and central effects of exposure to the Porsolt's test (FST) in mice of the C57BL/6 and DBA/2 strains. Behavioural effects are expressed as inactivity scores whilst central effects are expressed as percentage changes in dopamine release in comparison with controls (unhandled mice).
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GENES AND ALZHEIMER’S DISEASE

Benjamin Wolozin

INTRODUCTION
Alzheimer’s Disease (AD) is the most common neurodegenerative disorder affecting the elderly. In America, approximately 15% of the population over age 65 is afflicted by AD. AD has been detected worldwide in virtually every population that has been examined, from Japan to Germany and from Finland to South Africa. AD presents itself as a progressive loss of cognitive function. At first patients may complain of an inability to perform complex tasks related to work or finances, such as balancing a checkbook. As the disease progresses, the loss of cognitive function increases. Loss of short-term memory is one of the classic signs of AD, and in severe AD cognitive loss becomes so profound that even simple tasks such as eating or dressing become difficult.

The definitive diagnosis of AD can only be made by a post-mortem examination. The neuropathology of AD is characterized by the presence of two characteristic lesions, neuritic plaques and neurofibrillary tangles (Selkoe, 1996). Neither of these two pathological lesions is unique to AD, but an abundance of both of these lesions is pathognomonic of AD. Neuritic plaques are extracellular lesions. Many different proteins have been identified in neuritic plaques, but the most abundant, and probably most important, protein is β-amyloid (Aβ). The Aβ peptide is a small 40-43 amino acid protein that is very hydrophobic and therefore has a tendency to aggregate (Glenner and Wong, 1984). However, neuritic plaques also contain smaller amounts of several other proteins, which include apolipoprotein E, antichymotrypsin, complement C1,α, macroglobulin and an internal fragment of α-synuclein (Schmechel et al., 1993; Abraham et al., 1988; McGeer et al., 1990; Ueda et al., 1993).

Neurofibrillary tangles contrast with neuritic plaques because they are largely intracellular lesions (Wolozin et al., 1986). They are composed aggregates of microtubule-associated protein tau (Lee et al., 1991; Wolozin et al., 1986; Grundke-Iqbal et al., 1986). The study of the
neuropathology of AD has been intimately connected with advances in our understanding of the molecular genetics of other neurodegenerative diseases. Most of the proteins associated with Alzheimer neuropathology appear to have at least one corresponding mutation that leads to a related neurodegenerative disease in humans. The strong connection between neuropathology and genetics has led to an exciting synergism between the two fields, with discoveries in each field propelling advances in the other.

At first AD was considered to be largely a sporadic illness, disconnected from genetics. However, a steady stream of scientific discoveries has turned this initial view on its head, with the occurrence of AD being closely associated with genetic factors. Even before the identification of particular Alzheimer-related genes, early studies of AD suggested that there was a strong genetic component to the illness. One of the first studies examined the frequency of AD in first-degree relatives of patients with AD. This study found that in older Alzheimer patients, close to 50% of living first-degree relatives developed AD themselves (Mohs et al., 1987). This suggested that AD was following classic Mendelian patterns of autosomal dominant inheritance. However, these data were somewhat controversial because the sample size was much smaller in the older age groups, as patients died of other causes before they could develop AD.

The conclusion that genetic factors could cause AD became much clearer as investigators began to focus on a small number of patients who developed AD at an early age. About 5% of patients with AD develop the illness prior to age 55. The earlier age of death made it possible to develop a clearer picture of the mode of inheritance. Investigators studying these individuals observed that AD was transmitted as a classic autosomal dominant disorder with virtually complete penetrance. They also noted that, in these families, 50% of the first-degree relatives developed AD. In acknowledgment of the clear patterns of inheritance, these cases are now referred to as familial AD (FAD). The FAD patterns of inheritance allowed investigators to apply the techniques of molecular genetics to identify the specific genes causing AD in these families.

THE BIOLOGY OF AMYLOID PRECURSOR PROTEIN

Before examining these mutations causing FAD, it is worthwhile discussing the biology of amyloid precursor protein (APP) and Aβ because these are central to the pathophysiology of AD. As described above, researchers investigating the composition of neuritic plaques determined that Aβ was the major component. Purification of Aβ led to the identification of its sequence, and from this sequence investigators identified
a larger protein, APP, that has the same stretch of amino acids (Glenner and Wong, 1984; Kang et al., 1987).

APP is a large transmembrane protein that is ubiquitously expressed in the body (Fig. 1) (Kang et al., 1987). APP can be alternatively spliced and is therefore produced in three forms 695, 751 and 770 amino acids in length. The Aβ sequence is a small stretch of amino acids that extends from within the membrane spanning region into the extracellular domain. APP is normally cleaved and secreted, but the cleavage process can follow two different paths (Fig. 3) (Oltersdorf et al., 1989; 1990). In one pathway, APP is cleaved in the middle of the Aβ domain to generate a fragment termed APPsα that is secreted, and a C-terminal fragment that is internalized and degraded (Fig. 1) (Oltersdorf et al., 1990; Esch et al., 1990; Sisodia et al., 1990). The exact function of APPsα is not well understood, but it has been shown to be neuroprotective and to function as a protease inhibitor that regulates blood clotting (Van Nostrand et al., 1989; Furukawa et al., 1996; Barger and Mattson, 1996).

The second pathway leads to the formation of Aβ. In this pathway, APP is cleaved at both ends of the Aβ domain, leading to the formation of three different protein products. The extracellular portion termed APPsβ and Aβ are both secreted, while the C terminal fragment (termed P3) is internalized and degraded (Haass et al., 1992; Cai et al., 1993). The 16 amino acids that are present on APPsα but absent from APPsβ appear to be important for neuroprotection (Barger and Mattson, 1996). Thus, APPsβ does not exhibit as strong neuroprotective properties (due to the absence of these 16 amino acids) but is probably equally active in regulating blood clotting, because APPsβ contains the same protease inhibitory domain as APPsα (Van Nostrand et al., 1989).

The function of Aβ is much less clear. Like APPs, Aβ appears to play a role in blood clotting because it stimulates platelet aggregation (Wolozin et al., 1998). It also may play a role in regulating cholesterol uptake by cells, as it is bound by apolipoprotein E and α2-macroglobulin, two proteins that regulate cholesterol uptake (Strittmatter et al., 1993; Strittmatter et al., 1993; Du et al., 1997; Hughes et al., 1998). The ability of apolipoprotein E and α2-macroglobulin to bind Aβ turns out to be very important for the more classic forms of AD occurring in the elderly.

The most significant features of Aβ chemistry for Alzheimer's research is that Aβ aggregates and Aβ can be toxic at high doses (Yankner et al., 1990). Although Aβ is continually secreted by almost all cells, it is normally present in our body at only very low levels (less than 1 nM) (Esch et al., 1990; Haass et al., 1992). In AD Aβ accumulates in high
levels in neuritic plaques, leading to neurotoxicity and neurodegeneration, causing AD. Multiple factors control the accumulation of Aβ in AD, but the most basic is that Aβ is a hydrophobic peptide that has a strong tendency to aggregate in aqueous solution (Jarrett et al., 1993). Aggregation is a chemical phenomenon, and like all chemical reactions the rate is strongly influenced by a variety of factors (Jarrett et al., 1993; Jarrett and Lansbury, 1993). The two key factors are concentration and time. As the concentration of Aβ increases, the rate of aggregation increases. Neuritic plaques probably form in the brain because neurons make about three times more Aβ than other cell types, making the concentration in the brain higher than elsewhere.

AD is a disease of ageing in part because the aggregation process is very slow. The length of the Aβ produced influences the rate of aggregation. Most (95%) of the Aβ0 made by cells is 40 amino acids long (Aβ40). However, about 5% of the Aβ produced is 42-43 amino acids long because the protease that cleaves APP at the C-terminal site (termed γ-secretase) is imperfect (Shoji et al., 1992; Suzuki et al., 1994). Aβ42/43 is much more hydrophobic than Aβ40 and aggregates much more readily (Jarrett et al., 1993). For example, a 1 mM solution of Aβ40 requires about a day to aggregate, while the same solution of Aβ42 aggregates in minutes (Jarrett et al., 1993). The propensity of Aβ42/43 to aggregate rapidly is important because this peptide can also serve as a nidus for aggregation of Aβ40 by forming the first small aggregates, which is the rate-limiting step. Once these form, aggregation occurs much more rapidly by extending from the nidus (Jarrett and Lansbury, 1993). Thus, a solution of Aβ40 aggregates much more rapidly when spiked with a small amount of Aβ42. This same process appears to occur in the Alzheimer brain. Studies of neuritic plaques show that the core of the plaques often consists of Aβ42, while the bulk of the plaque is composed of Aβ40 (Iwatsubo et al., 1996; Nakamura et al., 1995).

Much of the genetics of AD can be explained by the simple concept that mutations in any protein that lead to increased Aβ aggregation, either by increasing production of Aβ or by changing the physical properties of Aβ, increase the risk of AD.

**MUTATIONS IN APP THAT CAUSE FAD**
Mutations in three different genes have been shown to cause most of the cases of FAD (Hardy, 1997). The first genetic mutation to be implicated in FAD was a mutation at amino acid 693 APP770 (Levy et al., 1990; Van Broeckhoven et al., 1990). This discovery exemplifies one of the classic patterns of research in AD, in which the neuropathology leads to an important discovery in molecular genetics. Following the
discovery of APP, researchers began examining the APP gene in diseases related to Aβ deposition. Success came with the identification of the APPQ693G mutation in a family with hereditary cerebral haemorrhage with amyloidosis, Dutch type. Soon after, another mutation was identified in a family with FAD in which a Val to Phe mutation at position 717 of APP770 caused the illness (Goate et al., 1991). This mutation leads to an autosomal dominant form of AD with an early age of onset. It turns out that mutations in APP are only rarely the cause of FAD; most cases of FAD are caused by mutations in a gene termed presenilin 1, which is discussed later. However, since the discovery of the APPF751V mutation, several different mutations in APP associated with FAD have been identified (Fig. 1) (Hardy, 1997).

The 717 position turns out to be a relative hot spot for FAD mutations in APP (Hardy, 1997). Multiple mutations leading to different amino acids have been identified at the 717 spot. A mutation associated with FAD has also been identified at position 692, and a double mutation associated with FAD has been identified at positions 670,1 (Citron et al., 1992; Cai et al., 1993). Finally, a mutation at position 692 has been associated with both FAD and a disease termed cerebral amyloid angiopathy (Hendriks et al., 1992). This dementing illness is characterized by the presence of extensive cerebral haemorrhage and dementia.

The positioning of these mutations highlights the importance of Aβ in AD. Each of these mutations occurs at positions in APP that are either in the Aβ domain or flanking the Aβ domain. Because of their proximity to the Aβ domain, all of these mutations affect Aβ production. The mutation at positions 670-1 occurs at the position where the enzyme β-secretase cleaves APP to generate the N-terminus of Aβ. This mutation enhances the β-secretase cleavage and increases total Aβ production three fold (Citron et al., 1992; Cai et al., 1993). The mutations at APP692 and 693 occur at the position where the enzyme α-secretase cleaves APP to generate APPα (Haass et al., 1994). These mutations both reduce the amount of APP cleaved by α-secretase and increase total Aβ production. They also increase the tendency of Aβ to aggregate (Wisniewski et al., 1991). Interestingly, the mutations at amino acids 692 and 693 of APP770 both greatly reduce the ability of Aβ to stimulate platelet aggregation (Wolozin et al., 1998). These mutant Aβ peptides accumulate extensively in the vasculature. This accumulation may interfere with platelet aggregation and cause the cerebral haemorrhage occurring associated with both hereditary cerebral haemorrhage with amyloidosis, Dutch type, and with cerebral amyloid angiopathy (Wolozin et al., 1998). The mutations at APP717 occur slightly distal to the γ-secretase site, which generates the C-terminus of Aβ. The 717
mutations all increase production of Aβ42 without affecting total Aβ production (Suzuki et al., 1994). Aβ42 normally constitutes 5% of total Aβ production, but in APP717 FAD it constitutes up to 15% of total Aβ production.

Because more Aβ or Aβ42 produces faster aggregation, each of these mutations speeds up the formation of neuritic plaques. Earlier neuritic plaque deposition means earlier AD and clearer genetics, hence FAD.

**MUTATIONS IN PRESENILINS THAT CAUSE FAD**

Although the mutations in APP were the first to be associated with FAD, most cases of FAD are caused by mutations in a different gene, termed presenilin 1 (PS1) (Sherrington et al., 1995; Clark et al., 1995; Hardy, 1997). In addition, a small number of FAD cases are caused by mutations in a homologue of PS1, termed presenilin 2 (PS2) (Levy-Lahad et al., 1995). PS1 and PS2 are proteins that have 8-10 transmembrane domains (Fig. 2) (Doan et al., 1996; Li and Greenwald, 1996). Whereas APP normally appears to play only a subtle role in cell biology, PS1 and PS2 have major roles in cell biology. PS2 plays a role in apoptosis, which is a form of cellular suicide (Wolozin et al., 1996; Vito et al., 1996; Vito et al., 1996). Mutations in PS1 also increase the vulnerability of cells to stress and increase apoptosis (Guo et al., 1997). In addition, PS1 is required for development and animals lacking PS1 die in utero (Wong et al., 1997).

The mutations in PS1 that produce FAD all increase production of Aβ42 (Scheuner et al., 1996; Citron et al., 1997). The mutations in PS1 and PS2 that are associated with FAD occur throughout the proteins (Fig. 2). These mutations appear to cause FAD because presenilins directly control cleavage of APP to produce Aβ. This is best understood for PS1. Although not a protease itself, PS1 is required for the action of proteases on APP. γ-Secretase, which is the protease that generates the C-terminus of Aβ, does not cleave APP in cells lacking PS1 (De Strooper et al., 1997). Similarly, α-secretase, which is the protease that generates APPα, is less active in cells lacking PS1 (Palacino et al., 1998). Thus, PS1 appears to be required for the interaction of secretases with APP. How the FAD mutations alter PS1 action and increase Aβ42 production is currently unclear. PS1 and PS2 have been shown to bind APP, and, as mentioned, PS1 is required for full α- and γ-secretase activities (Xia et al., 1997; Weidemann et al., 1997). Another clue comes from the observation that the PS1 mutations all reduce APPα production in addition to increasing Aβ42 production (Langer et al., 1998; Palacino et al., 1998). The reduced APPα production may reflect altered bind-
ing of PS1 to APP. If PS1 is required as a scaffold to couple secretases the altered binding properties of PS1 could cause γ-secretase to cleave at a slightly different position.

The increased production of Aβ42 produces faster neuritic plaque deposition and leads to early-onset FAD. Although all cases of PS1 FAD occur at very early ages (between ages 29 and 55), the exact age of onset does not correlate with the amount of Aβ42 generated. For instance, the L392V mutation produces a 3-fold increase in Aβ42 production and leads to FAD occurring by age 46, while the L286V mutation produces a 2-fold increase in Aβ42 production but leads to FAD at the same age, and the N141I mutation of PS2 increases Aβ42 3-fold but has a later age of onset (Citron et al., 1997). The fact that all PS1 mutations that are associated with FAD increase production of Aβ42 suggests that the effects of PS1 on Aβ42 production are sufficient to cause FAD. However, the exact age of onset may be modified by other aspects of PS1 biology. For instance, the exact age of onset tendency of FAD may be determined by the extent to which particular mutations in PS1 increase the sensitivity of neurons to cell stress and promote apoptosis.

TRANSGENIC MICE: PROOF OF CONCEPT

The research described above indicates that there is a strong association between FAD, neuritic plaques, increased levels of Aβ and mutations in APP, PS1 or PS2. In order to test whether these mutations actually cause neuritic plaque formation and FAD, researchers constructed transgenic mice carrying mutant forms of APP or PS1. The first experiments were done using APP constructs. These experiments proved to be exceedingly difficult. Humans develop FAD after 30 to 50 years, but mice live less than 3 years. This means that the production of transgenic Aβ must be high enough to increase the rate of Aβ deposition over ten-fold. After much effort a transgenic mouse that develops neuritic plaques was generated using an APPF717V construct (Games et al., 1995). The ability of transgenic APPF717V to induce neuritic pathology proves the link between APP, Aβ and neuritic plaques. This mouse begins to develop plaque pathology by 8 months of age and by 18 months of age 30% of its cortex is covered with neuritic plaques, which is even more extensive neuritic pathology than seen in an Alzheimer brain (20%) (Games et al., 1995).

Soon after, several other transgenic APP mice were generated showing neuritic pathology (Oster-Granite et al., 1996; Hsiao et al., 1996; LaFerla et al., 1996; Wyss-Coray et al., 1997; Sturchler-Pierrat et al., 1997). The neuritic plaques that develop in many of these transgenic
mice show a striking resemblance to neuritic plaques in the brains of Alzheimer patients. Despite being expressed throughout the brain, the transgenic neuritic plaques develop in the same regions as in the Alzheimer brain. Neuritic plaques develop in the hippocampus and cortex, but not in the thalamus, basal ganglia or cerebellum (Games et al., 1995; Sturchler-Pierrat et al., 1997). The transgenic neuritic plaques also show similar micropathology. For example, they are composed of Aβ but have a halo of activated microglia, which is similar to neuritic plaques in Alzheimer brains (Frautschy et al., 1998; Sturchler-Pierrat et al., 1997). This work clearly shows that changes in the metabolism of APP are sufficient to produce neuritic plaques.

Although these transgenic experiments show that there is a strong connection between neuritic pathology and Aβ, the transgenic animals all show considerable variability in other aspects of Alzheimer pathology. Only one of the transgenic APP mice develops neurofibrillary pathology (Sturchler-Pierrat et al., 1997). The amount of synaptic and neuronal loss is also highly variable between different transgenic mice. Two transgenic mouse lines show significant synaptic loss (Nalbantoglu et al., 1997; Sturchler-Pierrat et al., 1997). Some lines of transgenic mice develop cognitive deficits, like those of Alzheimer patients (Hsiao et al., 1996), but at least one of the transgenic mice that develops extensive neuritic pathology does not show cognitive deficits (Irizarry et al., 1997). As a result of these discrepancies, the connection between other aspects of Alzheimer’s disease and Aβ is more ambiguous.

Other genes that affect Alzheimer pathology probably cause the variation in pathology that occurs between the different transgenic mice. The strain of mouse used in these different transgenic lines often differs, and these differences clearly affect the resulting pathology. In other words, the genetic background of the animals appears to affect Alzheimer pathology (Carlson et al., 1997). A clear example of how other genes can affect Alzheimer pathology comes from the PS1 transgenic mice. Transgenic mice expressing human M146E PS1 show no pathology and are quite healthy. This is probably because mouse Aβ does not have the same tendency to aggregate as human Aβ. As a result, changes in the processing of mouse Aβ induced by the mutant PS1 construct do not lead to the accumulation of aggregated Aβ. However, the progeny of mice expressing human M146E PS1 and mice expressing APPF717V produce over 40 times as much Aβ42 and develop neuritic plaques as early as 4 months after birth (Borchelt et al., 1997; Holcomb et al., 1998). These results emphasize the strong connection between PS1 and Aβ, and provide a clear demonstration of how other genes can influence Alzheimer pathology. The ability of mutant PS1 to increase
the rate of Aβ deposition also provides a clear model demonstrating how these genes may produce early-onset FAD.

**GENES AFFECTING LATE-ONSET AD: APOLIPOPROTEIN E**

Whereas mutations that increase the production of Aβ cause FAD, genetic changes that increase Aβ aggregation predispose towards late-onset AD. Two different DNA polymorphisms have been identified that increase the risk of AD. One is in the ApoE gene and the other is in the α2-macroglobulin receptor. Both of these genetic changes differ from the mutations that cause FAD because they are common changes; hence they are termed polymorphisms rather than mutations. Three forms of ApoE protein are commonly present in the human population. ApoE2 has cysteines at amino acids 112 and 158, ApoE3 has a cysteine at amino acid 112 and an arginine at amino acid 158, and ApoE4 has arginines at both positions. In America, ApoE2, 3 and 4 are present at frequencies of 5, 75 and 20%, respectively; the frequency of the ApoE4 genotype varies from country to country, though. Multiple studies in multiple different countries have shown that the frequency of the ApoE4 genotype in patients with Alzheimer’s disease is twice as high as in the population at large (Roses, 1996). In America 40-60% of cases of AD are thought to be related to ApoE4 (Mayeux et al., 1998). This makes ApoE4 the single most important cause of AD identified to date. Studies of identified families that carry the ApoE4 polymorphism show that the risk for dying from AD is proportional to the number of ApoE4 alleles that they carry (Corder et al., 1993). Individuals homozygous for the ApoE4 allele are at greater risk of developing AD than individuals who carry one copy of ApoE4, and those heterozygous for ApoE4 are at greater risk than individuals who lack the ApoE4 allele. The ApoE4 allele also appears to increase formation of neuritic plaques and decrease the age of onset of AD, but does not appear to affect the interval between the age of onset and death (Gouras et al., 1997; Gearing et al., 1996; Gomez-Isla et al., 1996). Despite its effect on the age of onset, the correlation between ApoE4 and AD is less than 1. It is possible to have the ApoE4 polymorphism and not develop AD. Prospective studies indicate that the presence of an ApoE4 allele increases the risk of AD by about 30% (Gomez-Isla et al., 1996). Because of its lack of complete penetrance for AD, the ApoE4 polymorphism is termed a genetic risk factor rather than a genetically determined illness.

The mechanism by which ApoE4 increases the risk of AD appears to be by increasing Aβ deposition. All forms of ApoE bind Aβ in vitro and promote aggregation (Strittmatter et al., 1993a; 1993b). ApoE4 binds with the greatest affinity, followed by ApoE3 and then ApoE2. There is
some controversy about the binding of ApoE to Aβ. Binding of ApoE to Aβ varies depending on the conditions, and some groups have had difficulty showing this binding (Evans et al., 1995). In addition, some studies indicate that ApoE4 increases Aβ toxicity while others suggest that ApoE4 decreases Aβ toxicity. Studies of the neuropathology of AD show that ApoE accumulates in neuritic plaques, which suggests that ApoE does bind Aβ in vivo (Strittmatter et al., 1993; Gomez-Isla et al., 1996). Individuals with the ApoE4 genotype show increased ApoE deposition in neuritic plaques compared with those with the ApoE3 or E2 genotype (Gomez-Isla et al., 1996). This supports the hypothesis that the ApoE4 protein increases the rate of Aβ aggregation. The most direct support linking studies of ApoE to Aβ aggregation and plaque formation comes from studies of transgenic animals. Transgenic mice that lack ApoE (through knockout technology) but express the APPF717V gene show significantly less neuritic plaque formation (after 12 months) than animals that express both ApoE and APPF717V (Bales et al., 1997). This suggests that ApoE promotes neuritic plaque formation. Investigators are currently breeding animals that express ApoE3 or E4 and APPF717V in order to test the hypothesis that ApoE4 increases Aβ aggregation and neuritic plaque formation.

**GENES AFFECTING LATE-ONSET AD: α2-MACROGLOBULIN**

Recently, a α2-macroglobulin has also been shown to be an important risk factor for late onset AD (Marx, 1998). α2-Macroglobulin is a protease inhibitor that accumulates in neuritic plaques. Studies in vitro show that α2-macroglobulin binds Aβ, like ApoE (Du et al., 1997; Hughes et al., 1998; Du et al., 1998). α2-Macroglobulin shares another biochemical similarity with ApoE because it binds to the same receptor, the low-density lipoprotein receptor-related protein (Rebeck et al., 1995). Unlike ApoE, α2-macroglobulin inhibits Aβ aggregation and Aβ toxicity (Hughes et al., 1998; Du et al., 1998). Thus, α2-macroglobulin appears to have the opposite actions to ApoE. Molecular genetic studies have now shown that a polymorphism in the α2-macroglobulin gene correlates with increased risk for AD (Marx, 1998). This suggests that ApoE and α2-macroglobulin exist in equilibrium, normally balancing the tendency of Aβ to aggregate vs. remain soluble. Possibly, a loss of the ability of α2-macroglobulin to prevent Aβ aggregation increases the amount of neuritic plaque formation and the risk of AD.

**OTHER GENES CAUSING NEURODEGENERATIVE DISEASE**

In addition to the genes described above several other genes have been implicated in related neurodegenerative disorders, although not directly
in AD. Each of these related genes provides important clues about the pathophysiology of neurodegenerative diseases. In each of these diseases, aggregation of an important protein appears to initiate the disease process. Aggregation of tau protein is thought to generate neurofibrillary tangles in AD. Although no mutations in tau have been identified in AD, several recent studies have shown that mutations in tau do cause a rare form of dementia termed frontotemporal dementia (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). In this condition, a mutation at a transcriptional splice site appears to increase the amount of tau containing 4 microtubule binding repeat units made in the brain (typically the 3 repeat form is more abundant - Hutton et al., 1998). This 4 repeat tau has an increased tendency to aggregate. The increased tendency to aggregate may cause frontotemporal dementia. A mutation in a protein, termed α-synuclein, appears to cause some rare forms of familial Parkinson’s disease (Polymeropoulos et al., 1997; Kruger et al., 1998; Clayton and George, 1998). Although the effect of this mutation is unknown, α-synuclein has a well-documented tendency to aggregate. In addition, aggregates of α-synuclein have been shown to occur in most Lewy bodies, which are a neuropathological lesion common in Parkinson’s disease (Spillantini et al., 1998; Takeda et al., 1998). Aggregates of a fragment of α-synuclein are also common in neuritic plaques in AD, and α-synuclein accumulates in neurons in most neurodegenerative diseases. Finally, increased lengths of polyglutamine stretches in several different proteins appear to cause a number of other neurodegenerative diseases, including Huntington’s disease and spino-bulbar atrophy (Ross, 1995; Trottier et al., 1995; DiFiglia et al., 1997). The longer polyglutamine stretches have an increased tendency to aggregate, which appears to initiate the neurodegeneration. These polyglutamine expansions occur because of DNA slippage as the cell tries to replicate stretches of DNA-containing trinucleotide repeat units (Kunkel, 1993). Thus, in multiple different illnesses aggregation of proteins appears to interfere with cellular functioning and cause neurodegenerative illness.

**IMPLICATIONS FOR THERAPY**

The increasing knowledge about the cell and molecular biology of Alzheimer’s disease has stimulated a range of therapeutic strategies in AD. Despite the profusion of treatment strategies, an effective treatment for AD is still elusive. The first therapeutic strategy developed for AD was based on increasing cholinergic transmission by inhibiting acetylcholinesterase with compounds such as Physostigmine, Tacrine and Aricept (Rogers et al., 1998). This strategy is based on the observation that the
cholinergic innervation from the nucleus basalis of Meynert is dramatically decreased in AD. While these treatments have been shown to help some patients (about 35%), they do not prevent progression of the illness. A second treatment that has been evaluated clinically is the use of vitamin E to reduce free radical damage in AD. Clinical studies with vitamin E are encouraging, but indicate that vitamin E is not a cure (Sano et al., 1997). Large-scale trials showed that it reduced the progression of AD by about 50%. The development of more specific drugs to inhibit Aβ toxicity is a challenge because the high concentrations of Aβ present in neuritic plaques probably lead to binding to many different receptors capable of producing neurotoxicity. The result is that it is unclear which targets, if any, are the most important.

A promising line of pharmacotherapy for AD is the design of drugs to inhibit Aβ production. This treatment strategy is promising because the target and endpoint are readily identifiable. By monitoring drugs that inhibit specific secretases or reduce Aβ production pharmaceutical companies can identify potential drug candidates. These drugs have yet to enter clinical trials, but several pharmaceutical companies have reported promising candidates. Another major therapeutic strategy being studied is the use of anti-inflammatory agents to inhibit the progression of AD (McGeer and McGeer, 1996). Elderly individuals treated for two or more years with non-steroidal anti-inflammatory medications show a 39% lower risk of developing AD (Stewart et al., 1997). Finally, there is increasing evidence that estrogen can slow down the progression of AD. Women treated with estrogen replacement therapy have about 50% lower risk of developing AD (Henderson, 1997; Kawas et al., 1997). Thus, there are a number of promising therapeutic strategies for preventing AD; however, none of these strategies appears to be completely effective.

CONCLUSIONS
There is a general consensus that Aβ plays a central role in the pathophysiology of AD. Mutations or polymorphisms in different genes that affect Aβ metabolism increase the risk of AD (Fig. 3). These mutations appear to fall into two different classes: factors that increase Aβ production and factors that increase Aβ aggregation. Mutations in proteins that regulate Aβ production, such as APP, PS1 or PS2, all cause FAD by increasing Aβ production. Polymorphisms in proteins that bind to Aβ and regulate its tendency to aggregate, such as ApoE4 or α2-macroglobulin, increase the tendency of Aβ to aggregate. The central theme in much of this discussion is the phenomenon of protein aggregation. Mutations that increase aggregation of a number of other pro-
teins each lead to a slightly different neurodegenerative disease. As many biochemists know, proteins are unstable structures. They often have hydrophobic domains that can aggregate upon exposure to aqueous conditions. Genetic or biochemical changes that increase hydrophobicity or promote the exposure of these hydrophobic domains to water increase aggregation. This aggregation appears to be difficult for the cell to handle, perhaps because it stimulates free radical production or other cell death processes, such as apoptosis. Either of these outcomes could stimulate neurodegeneration.
FIGURE 1
The structure, processing and disease-related mutations in APP.

A. Structure of the αβ peptide. The 1-40 sequence is shown in bold lettering. The sites of mutations that are associated with disease are shown below.

B. The structure of APP. The protein is cleaved at three different sites. The proteases that cleave at these positions are termed α, β and γ secretase.

C. Processing of APP. Cleavage of APP takes place in the endoplasmic reticulum. At least two alternate cleavage pathways exist. One pathway yields APPsβ and Aβ, while the other pathway yields APPsα. In addition, both pathways generate a C-terminal fragment that is internalized and degraded.
FIGURE 2
The structure, processing and disease-related mutations in PS1. The PS1 protein has 8-10 transmembrane domains. It is normally cleaved as part of its processing at the beginning of the large loop after the 6th transmembrane domain (shown by the “x”). FAD mutations have been identified at multiple positions throughout the molecule.

Doan, et al, 1996
FIGURE 3
Pathophysiological mechanisms of AD. Aβ is synthesized from APP and secreted. With time it tends to aggregate and form neuritic plaques. The high concentration of Aβ associated with these plaques generates free radicals directly and also leads to binding to proteins that themselves either stimulate free radical production or stimulate cell death pathways. These proteins include the receptor for advanced glycation endproducts (RAGE), the low affinity p75 NGF receptor or a protein related to steroid hydroxylase enzyme that is termed ERAB. The end result of Aβ accumulation is neurotoxicity and neurodegeneration. Mutations in proteins that stimulate any part of this pathway appear to lead to AD. Thus, mutations in protein that increase production of Aβ40 or Aβ42, such as mutations in APP, PS1 or PS2, all increase the rate of Aβ deposition and lead to FAD. Polymorphism in ApoE4 or α₂-macroglobulin promote Aβ aggregation and lead to late onset AD. No disease related mutations have yet been identified in putative Aβ receptors.

Pathophysiological Mechanisms of AD
REFERENCES


