New order from neurological disorders

The neurological diseases described in this review supplement impact across a wide spectrum of the population. Here, their similarities and differences are highlighted, and common themes of the interactions between basic and clinical sciences for their understanding and treatment are explored.

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Neurological diseases affect the qualities that make the lives of humans so special, including memory, cognition, language, personality and skilled movements. The reviews in this supplement focus on the neurobiology of a variety of these diseases of the central nervous system (CNS), including cerebrovascular disorders, epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS) (Table 1). Some of these illnesses are common, whereas others are less frequent. For example, in the United States there are approximately 5 million elderly individuals with AD, and perhaps 500,000 persons with PD. Cerebrovascular diseases (stroke syndromes) are a major cause of morbidity and mortality in middle and later life. By contrast, MS chronically disables individuals in the prime of life, and epilepsy spans the range from newborns to the elderly, affecting about 1% of the population.

For each of these disorders, for the most part, only symptomatic treatments are available. Fortunately, over the past few years, significant advances have been made in clarifying the genetic features of some of these diseases, in developing new *in vitro* and *in vivo* model systems, and in understanding pathogenic mechanisms. Ultimately, these lines of research should lead to identification of new therapeutic targets and the design of new treatments that can be tested in model systems, and subsequently, in patients. Here I describe some of the important issues raised by studies of these disorders and attempt to show the great value of the critical interactions between basic and clinical neuroscience.

Clinical signs

For each disease, the clinical manifestations reflect the involvement of cells in different brain regions and circuits as well as the character and evolution of the biochemical and cellular abnormalities (Table 1).

In AD, the progressive memory deficits, cognitive impairments and personality changes are due to progressive dysfunction and death of neurons in the neocortex, limbic system, hippocampus and several of the subcortical regions of the brain¹. Similarly, in PD, the worsening motor signs — slowed movements, rigidity and tremor — are associated with progressive degeneration of dopaminergic neurons in the substantia

nigra². Certain types of epilepsy are associated with abnormalities in specific brain regions (for example, the medial temporal lobe or frontal lobe) and the clinical manifestations of seizures reflect loci of hyperexcitability in these neural systems³. Thus, signs of overactivity in the motor cortex manifest as uncontrollable movements of face, arm or leg, depending on the

region affected. In the case of the stroke syndromes, individuals with occlusions in a particular arterial territory suffer ischaemia in the region supplied by the compromised artery⁴⁻⁶. For example, occlusion of the upper branch of the left middle cerebral artery reduces blood flow to the left frontal cortex, and the resulting acute brain damage (infarct) is manifested by difficulties in producing (but not understanding) speech, as well as paralysis of the right arm. In MS, the acute, subacute or persistent clinical signs are related to the distributions of demyelinating lesions of nerve fibres; depending on the areas affected, patients can show visual impairments, paralysis and incoordination.

Thus, the clinical signs of the neurodegenerative diseases, epilepsy, stroke syndromes and MS are the consequences of the location and nature of lesions in the brains of individual patients. Moreover, the time courses of these illnesses are related to the nature and evolution of the cellular pathology in each disease. Thus, as a consequence of transient hyperexcitability, the signs of a seizure disorder are acute and paroxysmal; in cerebrovascular disease and in MS the clinical signs are usually acute or sub-acute, and, depending on the nature of the lesion, transient, recurrent or persistent; and in AD or PD, the symptoms are progressive, worsening inexorably over a

Table 1 Summary statistics of the neurobiology of diseases of the CNS

	Number of cases (in US)	Time course	Brain regions	Cellular pathology
Cerebrovascular disease	1.5 million new cases per year; third commonest cause of death	Acute, often persistent disability	Grey matter/white matter	Ischaemia/infarction; death of neurons; damage to axons
Epilepsy	~2.5 million cases	Paroxysmal	Neocortex, hippocampus, thalamus	Neuronal hyperexcitability
Alzheimer's disease	>5 million cases	Chronic, progressive	Neocortex, hippocampus, subcortical nuclei	Hyperphosphorylated tau in tangles; extracellular A β deposits; degeneration of subsets of neurons in specific brain regions
Parkinson's disease	>500,000 cases	Chronic, progressive	Substantia nigra	Degeneration of dopaminergic neurons; α -synuclein inclusions (Lewy bodies)
Multiple sclerosis	300,000 cases	Acute, subacute, remitting, chronic	White matter	Demyelination; loss of oligodendroglial cells

period of years.

Mechanisms and pathology of disease

The pathogenesis of these illnesses are as varied as the clinical signs. The mechanisms at work include: ischaemia/hypoxia; free-radical damage; necrosis and/or apoptosis; excitotoxicity; inflammatory and immune-mediated processes; impaired interactions between molecules; sequestration of essential molecules; formation of intra- and extracellular aggregates and fibrils; acquisition of toxic properties by mutant proteins; and pathological reorganization of neural circuits. For each disorder, there has been significant progress in clarifying these mechanisms.

In one form of epilepsy, immune-mediated damage to the brain is related to immunological attack of a subtype of glutamate receptor (GluR)⁷. This abnormality was discovered when rabbits, which were immunized with a fusion protein containing a part of GluR3 to generate anti-GluR3 antibodies, developed seizures and inflammatory lesions of the cortex which were reminiscent of the abnormalities identified in Rasmussen's encephalitis, a rare, progressive illness usually affecting children. But more common forms of epilepsy (for example, some temporal-lobe epilepsies) seem to be associated with reorganization of synaptic circuits, with the resultant activity in these aberrant circuits manifesting as seizures.

In contrast to these effects on the synaptic circuitry of the brain, the effects of MS are seen on electrical transmission along nerve fibres. In MS, by mechanisms that are still being established, the disease process targets and destroys oligodendroglial cells and their myelin sheaths⁸; axons are denuded of myelin and conduction velocities along these axons are reduced or blocked. In these foci of demyelination, axons are often spared and remyelination may occur with partial or complete resolution of clinical signs. However, in some cases, particularly in people with extensive areas of demyelination, there may be permanent damage to axons, making full recovery very unlikely.

Individuals with AD develop neurofibrillary tangles (paired helical filaments containing hyperphosphorylated tau) within the cell bodies or dendrites of neurons and neuritic plaques (swollen, tau-enriched neurites displayed around extracellular deposits of amyloid- β protein (A β)) in the affected areas. Once cleaved from the β -amyloid precursor protein (APP), the A β peptide is released from the cell into the local microenvironment (neuropil). Initially, there is a local increase in levels of A β ; eventually, the elevated levels of A β at these sites promote deposits of A β fibrils and trigger a cascade of events that damages

synapses, causes neurons to dysfunction and degenerate, and provokes responses of glial cells. People with familial and sporadic PD show intracytoplasmic inclusions (Lewy bodies) in neurons of the substantia nigra, and these bodies are enriched in α -synuclein^{9,10}. Significantly, the gene encoding this protein is mutated in some familial cases¹¹ (see below).

It has been more difficult to define the important but less well understood roles of tau in neurodegenerative diseases. In several of these illnesses, including AD, aberrant tau immunoreactivity is present in neural cells and processes in the cortex^{9,10}. Tau, a microtubule-associated protein, binds to tubulin and participates in microtubule assembly and stability; interference by mutations with the properties of tau could compromise events that are dependent on the normal functions of the neuronal cytoskeleton. To the great pleasure of scientists working in this area, tau is now the primary participant in its own set of diseases^{9,10,12-14}. For example, several studies now indicate that exonic and intronic mutations in *tau*, although not causing AD, do cause frontotemporal dementia associated with parkinsonism (FTDP) linked to chromosome 17, the locus for the *tau* gene¹²⁻¹⁴. The *tau* mutations in FTDP-17 impact on the levels of different tau isoforms (intronic mutations) and on the interactions of tau with microtubules (missense mutations)^{12,15}. Presumably, these abnormalities alter the properties of the cytoskeleton, leading to dysfunction and death of affected nerve cells.

Genetics

One of the most striking parallels emerging from recent studies of these different diseases is the extraordinary progress that has been made in identifying the specific genes that are significant risk factors for many of these illnesses and the ways in which proteins encoded by these genes are associated, directly or indirectly, with loss of functions or gain of adverse properties^{1,9,10,16,17}. In investigations of epilepsy in mice and humans, genetic determinants have been shown to be responsible for different types of seizures and are inherited as either autosomal dominants or autosomal recessives. An example of an autosomal dominant type of susceptibility is a form of epilepsy that can occur with a febrile illness; this syndrome has been linked to the presence of a point mutation in the β -subunit of a voltage-gated sodium channel. Autosomal dominant benign familial neonatal convulsions have been linked to mutations in two distinct, but related, calcium channels, whereas a form of autosomal dominant nocturnal frontal-lobe epilepsy is associated with mutations in the $\alpha 4$ subunit of the nicotinic cholinergic receptor.

In MS, there is clear evidence of clustering in families, enhanced risk among siblings, and increased frequency in some ethnic groups. There is also a higher risk among monozygote than dizygote twins. In some ethnic groups there is a clear susceptibility associated with the inheritance of certain histocompatibility markers (major histocompatibility complex on chromosome 6). Mutant genes can also cause stroke syndromes. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant disorder characterized by recurrent ischaemia in mid-life. In many families, the disease locus maps to the short arm of chromosome 19 at band 13 (19p13); patients with CADASIL have mutations in the *Notch3* gene⁴.

Similarly, advances have been made in showing how mutant genes cause subsets of cases of AD, PD, amyotrophic lateral sclerosis (ALS) and a variety of movement disorders including Huntington's disease (HD) and several types of spinocerebellar ataxia^{1,9,16-20}. In AD, several genes are known to be significant risk factors: those encoding APP and presenilin-1 or -2 (PS1, PS2) are mutated in some families with autosomal dominant disease; *ApoE* which, depending on the dose (one or two copies) of the *ApoE4* allele, acts as a time-dependent susceptibility gene; and, possibly, $\alpha 2$ macroglobulin which, as a deletion mutant, may increase the probability of dementia in later life¹. With regard to genetic forms of PD, studies in several families have shown that the disease can be caused by at least two missense mutations in α -synuclein^{9,11}, an abundant synaptic protein whose normal functions are not well defined. HD and several of the inherited ataxias are caused by expanded trinucleotide repeats (coding for elongated stretches of glutamine) in specific genes including those encoding huntingtin, ataxin-1 and atrophin-1. Mutations in the gene encoding superoxide dismutase 1 are one cause of autosomal dominant ALS.

Animal models

Over the past few years, with the advances in genetics and in transgenic/gene targeting strategies, there has been great progress in modelling some of these genetic neurological diseases^{1,9,16-21}. For example, transgenic strategies have been used to model ALS, HD and several inherited ataxias¹⁷⁻²⁰. Taking advantage of the information about the mutations in APP and PS1, investigators have created mutant APP and APP:PS1 transgenic models of A β amyloidogenesis^{1,21}. The APP-mutant mice develop memory deficits (ref. 21, and A. Markowska, D.L.P. and D. R. Borchelt, unpublished data), which occur before the

onset of overt deposition of A β in the hippocampus and neocortex; at later stages, neuritic plaques, comprising amyloid deposits and neurites (which represent altered axons and synaptic terminals), are evident in these regions.

The mechanisms leading to the synaptic abnormalities underlying the impairments in memory have not been defined. This exciting area of research has implications not only for AD, but perhaps also for some of the other abnormalities in cognition and memory that may occur in the elderly. Significantly, although these mice are excellent models of A β amyloidogenesis, in general, they do not show overt neurofibrillary pathology^{9,10}; perhaps the introduction of wild-type human tau into APP transgenic mice showing A β deposits will facilitate development of tangles in neurons.

Although several groups have made mice that overexpress either wild-type α -synuclein or tau, so far there are no published results showing that these lines of mice develop clinical signs and pathology similar to that occurring in the α -synuclein- or tau-related diseases. However, because the overexpression of mutant transgenes has worked so well for other inherited diseases, it is likely that lines of mice expressing mutant α -synuclein or mutant tau will develop some of the clinical, pathological and chemical features of the cognate human diseases.

New technologies

Recently developed *in vitro* and *in vivo* model systems are crucial for studies designed to delineate the great variety of pathogenic mechanisms occurring in diseases of the CNS. New technologies will be essential for molecular analyses of these processes. The development of laser capture microdissection and the molecular studies of samples of neural tissue obtained with this approach^{22–25} should be very helpful in allowing investigators to examine the biological measures at the level of individual cell populations. This approach enables investigators to overcome problems previously encountered owing to the heterogeneity of CNS tissues (M. Lee and S. Bova, personal communication). Being able to select out samples of specific cells will facilitate, for individual cell types, studies of gene expression, analyses of the differences in amounts of transcripts and proteins, and identification of modifications of proteins. This approach will shed light on the molecular players in some of the pathogenic cascades occurring in selectively vulnerable cell populations, which in turn should help identify new therapeutic targets.

Prospects for therapy

In the past, relatively few treatments for

these illnesses have been directed selectively at disease mechanisms, but more recent strategies have begun to focus on pathogenic processes^{2,3,6,8,26}. Cerebrovascular disease is a common illness linked to a variety of risk factors, including hypertension, hypercholesterolaemia and diabetes, and the prevalence of stroke can be reduced by treatments that ameliorate these conditions. More recent efforts have shown that acute stroke can be treated with thrombolytic agents. Moreover, excitotoxic cell death, which occurs in cerebrovascular disease and is associated with glutamate toxicity and changes in calcium levels, may be modified with neuroprotective approaches. The spectrum of potential therapeutic targets, which can be examined in a variety of stroke models, may soon be extended to include other processes that injure brain tissues during ischaemia, including apoptosis⁶.

The development of animal models of the different types of epilepsy (caused by genetic, immunological or inflammatory processes or by damage-associated reorganizations of circuits), whether experimentally induced or genetically engineered, should allow analyses of the physiological and cellular events leading to seizures in animals³. The results of such studies should help to identify some of the pathways leading to the development and propagation of seizures and, eventually, to the testing of new antiepileptic drugs.

With regard to AD, because of the significance of A β amyloidogenesis, many investigators have speculated that blocking γ -secretase, the ultimate activity that liberates A β peptide, will prevent this process, reduce the A β burden and attenuate A β -related downstream pathogenic events¹. Several companies have compounds that show some efficacies in blocking γ -secretase activity *in vitro*. With the availability of mutant-APP transgenic mice^{1,21} that show memory impairments and A β deposits, it is now possible to test the effects of these reagents on performance on behavioural tasks and on amyloidogenesis *in vivo*. Similarly, in PD, new surgical approaches, neuroprotective and cell-replacement strategies, and gene-transfer therapies have been developed and each of the approaches are being assessed in model systems^{2,26}.

Conclusions

As this excellent collection of reviews illustrates, substantial progress has been made over the past few years in understanding a variety of devastating neurological illnesses. Model systems are now being used for molecular and cell biological studies of pathogenic mechanisms and for identification of new therapeutic targets. The efficacies of new drugs can be examined in these models. Subsequently,

selected compounds will be tested in clinical trials, and, if effective, will be used rapidly to treat the many patients suffering from these crippling diseases of the nervous system. □

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