ating the meaning of nouns, there is activity in a midfrontal area called the anterior cingulate gyrus, but after practice with a list, the activity is reduced and returns when a new list is given. Areas in and around the anterior cingulate are active during tasks that involve words, spatial objects, or motor learning and do not seem to be specific to any particular domain of activity (*4*, *10*). During generation of novel word meanings, in addition to the anterior cingulate, two left cortical areas known to be related specifically to word processing are also activated. In general, attentional networks appear to comprise a number of subcortical areas that may differ depending upon whether their function is to orient to sensory stiumli, maintain the alert state, or carry out a variety of executive operations (*3*). These anatomical results open up the possibility of progress in many topics: what brain structures are involved in awareness, in voluntary control of information storage, in motor responding, and in maintaining current goal states (*11*).

The new brain imaging techniques have revealed a convergence of what we see and what we think; thinking about a telephone activates some of the same brain areas as seeing a telephone. Specific brain areas are activated when people are presented with sensory stimuli. These activations are in the areas one would expect on the basis of the type of sensory stimulation involved (*4*). For example, activations have different locations within the primary visual cortex when visual events are presented at different retinal locations, as would be expected from many visual mapping studies (*12*). When the visual stimuli utilize color or motion, prestriate areas become active that correspond to what would be expected from studies of cellular recording in monkeys (*4*).

When subjects are instructed to attend to color or motion, there is an increase in activation in the same prestriate areas that process these sensory dimensions (*4*). Moreover, if subjects are asked to create a visual image based on their remembered knowledge of a visual form, areas in the visual system also show increased activation (*13*). These findings support the general idea that processes initiated internally from instructions can activate the same sensory areas where these computations are performed on actual sensory events. The finding that imagery and perception share some of the same neural machinery was anticipated by many cognitive theories, but the topic had been subject to seemingly endless dispute before this evidence from brain imaging methods was available (*14*).

Understanding of how voluntary attention affects the activity within sensory-specific cortex requires an analysis of the brain circuits by which higher level instructions influence sensory areas. Because of the relatively long delay between neural changes and the changes in blood vessels, it is useful to relate the functional anatomy method of PET and MRI to time-dependent measures involving surface or depth recording of electrical or magnetic potentials (*6*). These methods allow a precise measurement (in milliseconds) of changes between experimental and control conditions and can be used for tracing the circuitry of a particular mental activity. Methods of recording electrical and magnetic activity outside the head have improved in recent years and we now know that there are specific generators of these signals. This knowledge has already helped to spur developments relating the two types of measures (*15*).

The ability to study the human brain by physiological methods is likely to transform our understanding of what the brain does. If the neural systems used for a given task can change with 15 minutes of practice as in the figure, how can we any longer separate organic structures from their experience in the organism's history? We must be able to trace the changes in the brain that occur with experience. Individual genetic makeup and learning together shape brain structure. We now have methods to understand how this takes place and what it means for the limits of human potential.

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Molecular Genetics of Neurological Diseases

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Over the past century physicians, primarily neurologists, have meticulously collected and categorized families with Mendelian-inherited neurological syndromes. The phenotypes of these disorders, of which there are more than 1000, have provided the framework in the past decade for rapid advances in identifying genes that cause them. To date, mutations of genes have been characterized in more than 40 disorders affecting the central and peripheral nervous system, and precise chromosomal localizations have been determined in many more (*1*). Genes have been identified for Duchenne muscular dystrophy, neurofibromatosis type 1 (NF1), Huntington's disease (HD), familial amyotrophic lateral sclerosis, neurofibromatosis type 2 (NF2), and for several muscle disorders including myotonic dystrophy, hyperkalemic periodic paralysis, and malignant hyperthermia.

These discoveries have led to several new concepts that have caught the attention of human geneticists. Perhaps the most important is the recognition that mutations consisting of unstable trinucleotide repeats within exons or in nonexpressed regions near a gene correlate with the disease phenotype (*2*). To date, five neurological disorders, several of which appear first at mid-

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to late life, fall into this category (see table): myotonic dystrophy, in which the number of CTG repeats at the 3' end of the gene encoding myotonin protein kinase correlates with disease severity (*3*); fragile X syndrome, a common cause of mental retardation in males, where CGG repeats near the 5' end of the gene *FMR-1* correlate with the disorder (*4*); spinobulbar muscular atrophy (Kennedy syndrome), in which CAG repeats occur in the androgen receptor gene (*5*); HD, a disorder of mid-life onset in which CAG repeats occur in the 5' region of the identified gene (*6*); and spinocerebellar ataxia type 1, in which CAG repeats have been found in a gene with a partially identified sequence (*7*). These discoveries have spurred new strategies for searching the genome for other mutations characterized by such repeats (*2*).

In the case of HD, the culmination of the long search for the gene identified a novel protein of 348 kilodaltons (*6*). The CAG repeats vary in the normal population from 9 to 37 copies, whereas on HD chromosomes the length ranges from 37 to 121 copies (*8*) (see table). There is a correlation between the number of repeats and the age of onset of the illness (*9*). In both HD and myotonic dystrophy, "borderline normal" numbers of repeats (called "premutation") can expand in the next generation to a size associated with disease. Furthermore, as successive generations show increased repeats, the disease often appears earlier and is more severe—a phenomenon defined clinically as anticipation. The discovery of the HD gene will lead to more precise presymptomatic testing, although the uncertainty of the significance of repeats in the borderline range will be a confounding factor.

The defect in DNA replication underlying the instability of trinucleotide repeats is unknown. Irregularities of replication can occur during meiosis (where all daughter cells are presumably affected) or at subsequent mitoses (where somatic cell variations can follow). This has provided an explanation for variations in expression and penetrance that characterize genetic disorders. For example, in myotonic dystrophy, variable expression of the expanded trinucleotide repeats in somatic tissues may account for the widespread phenotypic variations observed clinically.

The gene defects in certain rare neurologic developmental disorders have led to the discovery of new proteins with key roles in neuronal migration. In Kallmann syndrome, an X-linked recessive disorder that is characterized by hypogonadotropic hypogonadism and abnormalities of the sensation of smell (anosmia), the defective gene (*KALIG-1*) encodes for a protein with homology to neural cell adhesion molecules (*10*). Mutations in this unique protein cause defects in the migration of olfactory sensory neurons into the olfactory bulb and concomitantly block normal migration of gonadotrophin releasing hormone (GnRH) neurons from the olfactory placode into the hypothalamus. The resulting syndrome combines a defect in hormonal regulation with a defect in a primary sense, that is, smell. In another disorder, Miller-Dieker syndrome, the cerebral cortex fails to develop normal convolutions of gyri and sulci, a condition called lissencephaly (*11*). The responsible gene encodes a unique protein similar in sequence to G protein signal transduction molecules. Failure of normal expression of this gene product is believed to account for a widespread defect in neural migration to the cerebral cortex.

Elucidation of the structure of muscle sodium channels provided the backdrop for molecular definitions of a number of skeletal muscle disorders characterized by episodes of paralysis and myotonia (abnormal sustained muscular contractions) (*12*). Unusual phenotypes can now be segregated by location of point mutations in the human sodium channel. Yet other disorders of muscle contraction are caused by mutations in muscle chloride channels (*13*). In the latter instance, both autosomal dominant and autosomal recessive phenotypes have emerged.

The analysis of these familial patterns of illness and of the genetic mutations that cause them has given rise to new theories of phenotypic characterization, changing dramatically our concepts of the mechanisms underlying Mendelian inheritance. It is recognized now that heterozygous individuals can show the autosomal dominant phenotype, even though the normal allele appears to be fully expressed. Take the case of autosomal dominant hyperkalemic periodic paralysis, in which half of the sodium channels are mutated and defective in sodium flux. Apparently, this abnormality is sufficient, in the face of rising extracellular potassium, to elicit changes in excitability that become symptomatic. Hence, a subset of mutated channels (approximately 50%) can cause sufficient changes in membrane excitability to cause paralysis. In other disorders like autosomal dominantly inherited retinitis pigmentosa, which is due to mutations in rhodopsin, the accumulated defect in signal transduction, over decades, leads to degeneration of photoreceptor cells (*14*).

In NF1 and NF2, loss of tumor suppressor genes are responsible for the autosomal dominant phenotype. In NF1, a second mutation ("second hit" as described by Knudson) can be variably expressed in somatic cells, giving rise to variable expression of the disorder (*15*). Prior notions that autosomal dominant disorders are caused simply by partial loss of normal protein function and autosomal recessive disorders by total loss, no longer explain these inheritance patterns.

There has been great interest in the genetic basis of Alzheimer's disease. Familial Alzheimer's disease (FAD) shows nonallelic heterogeneity and the same phenotype, differing only by age of onset, can be caused by at least four different genetic loci. The linkage of early-onset FAD to chromosome 21q, which led to identification of mutations in the amyloid precursor protein (APP) gene, is a rare and unusual cause of the familial disorder (*16*). Fewer than a dozen families have been identified worldwide with mutations at this site. A second chromosomal locus for early-onset FAD has been identified on chromosome 14q24.3 (*17*), a region close to several candidate genes: the oncogene *c-fos*, the gene for the heat shock 70-kilodalton, and the transforming growth factor–β gene. More likely, an as yet unidentified gene will be found to be the cause. This site accounts for over 70% of FAD families of early onset. A third locus for late-onset FAD has

NEUROLOGIC DISORDERS DUE TO UNSTABLE TRINUCLEOTIDE REPEAT

Disorder	Chromosome locus	Trinucleotide repeat	Normal range	Disease range
Spinobulbar muscular atrophy	Xq21.3	CAG repeat of androgen receptor gene	13–30	30–62
Fragile X syndrome	Xq27.3	CGG repeat of *FMR-1* gene	6–54	50–1500
Myotonic dystrophy	19q13.3	CTG repeat of cAMP-dependent muscle protein kinase	5–37	44–3000
Huntington's disease	4p16.3	CAG repeat of HD gene	9–37	37–121
Spinocerebellar ataxia type1	6p24	CAG repeat of gene of unknown function	25–36	43–81

been identified on chromosome 19q (*18*). The recent demonstration that this locus is close to the gene for apolipoprotein E, which is involved in cholesterol transport into brain, has led to an interesting hypothesis that the apolipoprotein E4 allele may have a causative role in late-onset Alzheimer's disease, both inherited and sporadic forms (*19*). Evidence for a fourth locus for Alzheimer's disease among families of Volga-German descent arises from exclusion of chromosomes 14, 19, and 20 in these pedigrees (*1*). In sum, it seems likely that there are multiple causes of Alzheimer's disease, and perhaps only a minority have defects in the metabolism of APP.

Mutations in the prion protein are now known to explain three neurologic disorders, all inherited as autosomal dominant traits: Gerstmann-Straussler-Scheinker syndrome, familial Creutzfeldt-Jakob disease, and fatal familial insomnia (*20*). These disorders, which occur in mid- to late life, are associated with the rapid onset of neurological signs and symptoms resulting in death within a period of 1 to 3 years. These mutations have important implications for understanding pathologic processes that are both infectious and genetic.

Finally, the causes of neurological diseases are not limited to mutations in nuclear DNA. The identification of mitochondrial DNA mutations, giving rise to maternally transmitted diseases, has opened an entire new area for the analysis of complex multisystem diseases (*1*). Defects in mitochondrial DNA give rise to Kearns-Sayre syndrome, Leigh syndrome, Leber hereditary optic neuropathy, myoclonic epilepsy and ragged red fiber disease, and mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes. Furthermore, recent evidence suggests the possibility that increasing frequency of mutations in mitochondrial DNA with age may contribute to the pathologies of Alzheimer's and Parkinson's disease.

These advances in the diagnosis of genetic neurologic disorders present new challenges for the practicing physician. Presymptomatic genetic testing, developed with great care for HD, has suddenly become possible for a myriad of other disorders. The ethical concerns related to these challenges have not been adequately addressed and will remain a problem for physicians and families. Who will pay for genetic testing? Who can guarantee confidentiality? Can insurance carriers request that genetic testing be accomplished before insurance is underwritten? Who will provide the background evaluation to determine adequate understanding by the patient of the implications of genetic testing? Who will guide the patient through the difficult choices that sometimes must be made about fetal survival after prenatal testing?

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Excitement About Calcium Signaling in Inexcitable Cells

James W. Putney Jr.

Calcium ions act as intracellular messengers that control the functions of cells in many living systems. Traditionally, calcium signaling has been divided into separate categories: studies focusing on electrically excitable cells, such as nerve and muscle, and studies focusing on electrically inexcitable cells, such as epithelial or blood cells. Both excitable and inexcitable cells utilize calcium sequestered in cytoplasmic storage compartments for signaling, but excitable cells rely on a "calcium-induced calcium release" (CICR) mechanism (*1–3*) while, for inexcitable cells, the predominant mechanism for release is triggered by a diffusible messenger, inositol 1,4,5-trisphosphate (IP_3) (*4*). Although signaling in both types of cells is influenced by plasma membrane calcium channels, the channels in the plasma membrane of inexcitable cells apparently are not regulated by membrane potential and their pharmacology is different from that of the voltage-sensitive calcium channels of excitable cells. Recent discoveries, however, have revealed that calcium signaling mechanisms in excitable and inexcitable cells are more similar than previously suspected.

In electrically inexcitable cells, calcium signaling typically is a biphasic process (*5*). Neurotransmitters and hormones stimulate an intracellular organelle to release stored calcium into the cytoplasm, and this release is followed by entry of calcium into the cytoplasm from the extracellular space. The first phase of the calcium signal is attributable to IP_3, a small, polar molecule (*4*). Occupation of plasma membrane receptors activates enzymes that generate IP_3 from plasma membrane phospholipids. The liberated IP_3 then diffuses to specific receptors on an intracellular calcium-storing organelle, either the endoplasmic reticulum or a specialized portion of it called the "calciosome" (*6*). The IP_3 receptor is a ligand-activated, calcium-selective channel. The binding of IP_3 increases the probability of channel opening, which allows calcium to flow into the cytoplasm (*7*). The second phase of the calcium signal likely does not result from the direct action of either a plasma membrane receptor or inositol phosphates, but instead appears to operate through a "capacitative" mechanism (*8, 9*). In capacitative calcium entry, the empty calcium-storing organelle produces a retrograde signal that activates calcium influx across the plasma membrane. An electrical current associated with this entry has been characterized and designated I_{CRAC} (*10, 11*), meaning "calcium release–activated calcium current." The missing link for the capacitative calcium entry theory has been the identification of the signal from the intracellular calcium store.

Enticing clues about the identity of this signal come from the recent work of Randriamampita and Tsien (*12*). A diffusible messenger that is released from intracellular compartments in activated Jurkat cells (a T-lymphocyte tumor cell line) stimulates calcium influx across the plasma membrane in macrophages, astrocytoma

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