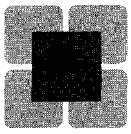

Experimental limbic epilepsy: models, pathophysiologic concepts, and clinical relevance

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■ Complex partial seizures originating in the temporal lobe are one of the most common types of seizures in patients with epilepsy. They are frequently intractable to medical treatment and are increasingly considered for surgical therapy. These seizures are often associated with focal epileptogenicity in limbic structures (amydgala and hippocampus) or with rapid spread of seizure activity to these areas. Much research is being undertaken to better understand this disorder and to develop more effective approaches to diagnosis and treatment. Experimental work in animals has contributed to the understanding of epileptogenesis, the interictal state, and the homeostatic mechanisms that limit seizure activity.

□ INDEX TERMS: EPILEPSY; LIMBIC SYSTEM; DISEASE MODELS, ANIMAL □ CLEVE CLIN J MED 1991; 58:521–530

What predisposes a patient to limbic epilepsy? What increases vulnerability or resistance to it? How does a limbic epileptic focus mature, and how do seizures express themselves from it? What relationship does the focus have to the surrounding brain? How are seizures contained? Current diagnosis and treatment are based on the theories developed by researchers to answer these questions.

Animal models have been essential to the formulation and testing of hypotheses related to limbic epilepsy. In this report, we review commonly used experimental models of limbic epilepsy. We outline the questions addressed by each model, the contributions of the model to current knowledge, and areas of current investigation. We synthesize current concepts about the pathophysiology of limbic epilepsy and outline future directions for research and clinical application.

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BACKGROUND

Epilepsy is one of the most common neurological disorders. It is estimated that about 10% of all Americans will experience at least one seizure during their lifetime.[1] Approximately 1% suffer from spontaneously recurrent seizures, or epilepsy.[2]

Epilepsies are classified primarily as *generalized* or *partial*. Partial (also called focal or local) epilepsies can have simple or complex symptoms, with or without secondary generalization of seizures.

In focal epilepsy, regional brain dysfunction (associated with previous injury or existing structural

TABLE
MODELS OF EXPERIMENTAL FOCAL LIMBIC EPILEPSY

Model	Animal	Mode of induction	Latency period	Duration of seizures	Seizure characteristics	Pathologic lesion(s)	Applications
Congenital limbic seizures	Rats; mice	Spontaneous; stimulus induced	Variable	Variable	Stereotyped complex partial	None (?)	Genetics of epilepsy; pharmacotherapy; neurotransmitters; electro-clinical correlations
Kindling	Rats; mice; higher animals (less prone)	Electric stimulation	Long (months)	Variable stimulus dependent	Variable (depend on kindled structures)	None (?)	Secondary epileptogenesis; perpetuation and extinction of epilepsy; electro-clinical correlations
Alumina cream	Rats; mice; cats; dogs; others	Bitemporal intraparenchymal injection	Long (months)	Variable; unpredictable	Variable	Large (focal)	Species susceptibility; lesion-focus relationships
Kainic acid	Rats; mice; others	Intrahippocampal; intra-amygdala; intraventricular; intraperitoneal; subcutaneous; intravenous	Short (hours, days)	Variable; depends on mode of induction	Stereotyped complex partial and generalized	Small but diffuse; multifactorial	Pharmacotherapy; neurotransmitters; metabolism; electro-clinical correlations; "excitotoxicity"
Tetanus toxin	Rats	Intrahippocampal; intra-amygdala	Intermediate (2-3 weeks)	2-4 weeks	Stereotyped complex partial	Small focal and self-limited at injection site	Same as Kainic acid (but without confusion of distant lesions); maturation, expression and extinction of epileptogenesis

lesions) leads to localized synchronous excitatory (epileptiform) neuronal activity, which can propagate to more distant brain regions. The clinical manifestations of a seizure reflect specific electrophysiologic events associated with the genesis and propagation of neuronal epileptiform activity. The relationships (possibly causal) between structural lesions and epileptogenicity are poorly understood. Also, there are many theories about the containment or perpetuation of seizures, functional inhibition in the surrounding brain, and secondary epileptogenesis—the concept that seizures themselves cause new epileptogenicity in nearby and remote regions of the brain ("seizures beget seizures").

Complex partial seizures originating in the temporal lobe are the most common seizure type in approximately 40% of patients with epilepsy[3] and tend to be more refractory to medical treatment than most other types of seizures.[4] They are associated with focal epileptogenicity in limbic structures (amygdala and hippocampus) or with rapid spread of seizure activity to these areas. They are frequently associated with hippocampal sclerosis, which results from perinatal hypoxia, trauma, or febrile seizures or other neurologic insults. Adjacent tumors or other structural lesions of the temporal lobe may also give rise to hippocampal epileptogenicity.

ANIMAL MODELS OF LIMBIC EPILEPSY

Experimental animal models of chronic focal epilepsy are developed by creating an epileptogenic lesion in the brain of an experimental animal. Different techniques have been adopted over the years to achieve this objective. Some strains of animals are prone to develop epilepsy, spontaneously or with minimal "induction."[5] Epileptogenic lesions have been produced by applying a variety of substances—such as cobalt,[6] tungstic acid,[7] penicillin,[8] alumina cream,[9] and zinc sulfate[10]—directly to the brain of "normal" animals. Focal tissue lesioning can also be achieved by a variety of mechanical techniques, including freezing.[11] A wide variety of animal species has been used in these experiments, including

primates,[12] dogs,[13,14] cats,[15–17] rabbits,[9] rats,[18–22] mice,[23] reptiles, amphibians, and invertebrates.[24,25] While various brain regions have been investigated, limbic structures have been the subject of intense interest in the attempt to reproduce the complex partial seizures which are so prevalent and intractable in humans.

Experiments in smaller animals, including rats and mice, have proved especially fruitful because they have relatively large and well-developed limbic structures. The morphological organization and electrophysiologic and biochemical peculiarities of limbic structures are consistent across phylogenetic lines, despite the smaller relative size of these structures in higher animals. Furthermore, limbic structures are thought to play a role in learning and memory across species, although they are more intimately associated with olfaction in lower animals and with emotion and cognition in higher primates and man. Following is a brief overview of five widely used animal models of focal limbic epilepsy (*Table*).

Genetically prone strains

There are strains of mutant or double-mutant rats and mice which are prone to seizures. Some strains exhibit spontaneous complex partial seizures. In other strains, repetitive motion, such as tossing or rocking, can precipitate such seizures.[5]

Electroencephalographic information about seizures in these animals is limited. However, these models have been used extensively in drug testing[26] and genetic[27] studies. They are also amenable to a host of investigations on metabolism and neurotransmitter alterations.[28] They may hold the key to understanding genetic factors in epilepsy and its developmental neurobiology.

Although some strains exhibit typical complex partial seizures of limbic origin, there is often no localized focus of brain dysfunction. In this regard, the models are not ideal for investigations exploring the relationship of the epileptic lesion to surrounding brain or the maturation and expression of the epileptic focus.

'Kindling'

In 1961, Delgado and Sevillano observed that repeated electrical stimulation of the cat hippocampus produced seizures.[29] In 1969, Goddard et al[30] reported electroencephalographic epileptiform discharges and clinical automatisms and convulsions in several animal species subjected to repeated administration of low levels of electrical current to subcortical regions. They discovered that repeated administration of an initially subconvulsive stimulus progressively intensifies interictal epileptiform neuronal activity and culminates in a clinical seizure they coined the term "kindling" to describe this phenomenon.

The exact mechanism underlying kindling is not known. One hypothesis postulates structural modification and potentiation of preexisting synaptic configurations in *groups* of neurons leading to synchronous epileptic discharge.[31] Another hypothesis suggests that kindling may influence *individual* neurons by modifying ionic channels and pump mechanisms, leading to neuronal destabilization and epileptic burst responses that integrate into seizures when a critical number of such neurons fire simultaneously.[32]

Kindling is probably the most widely used technique to model chronic focal epilepsy.[33] It is particularly attractive because the technique does not cause extensive brain damage, as other techniques do in many other models.[34] It can be used with success in many species, including rats,[35] mice,[36] monkeys,[30] and baboons.[34] Finally, the ability to kindle seizures in multiple brain sites permits investigators to select the most appropriate site for the experimental goals at hand. These features of the kindling model allow the investigation of vulnerability to epileptogenesis across phylogenetic lines and in various brain regions.[37]

The kindling model has been instrumental in correlating epileptic symptoms with electrical activity in the brain and in elucidating patterns of ictal propagation. The activity of neurotransmitters, such as gamma- aminobutyric acid (GABA),[38] somatostatin,[39] and opioid peptides,[40] has been studied extensively in this model. The kindling model has revealed that repeated burst discharges from an epileptic focus may permanently reorganize neuronal connections both locally and at a distance. This results in a progressive increase in the size of the epileptic zone as well as establishment of secondary foci at distant sites.[41] Thus, the kindling model has provided the framework for the theory of secondary or remote epileptogenesis, which explains many experimental and clinical observations consistent with the concept that "seizures beget seizures."

The disadvantages of kindling include the length of time required to develop an epileptic focus[42] and the inconsistency of epileptogenesis in certain structures.[30,37] Also, exogenous electrical stimulation over long periods may itself induce certain epiphenomena which are unrelated to epileptogenesis. Moreover, lengthy electrical stimulation is certainly not necessary to produce limbic seizures clinically.

Alumina cream

Alumina cream was first used to produce focal epileptogenic lesions in experimental animals in the early 1940s.[9] In most of the early studies, alumina cream was applied to the sensory motor cortex and thus did not focus on inducing limbic seizures.[43–45] However, later attempts were made to administer alumina cream into mesial temporal lobe structures to induce temporal lobe seizures.[46,47]

The technique was plagued with drawbacks: the resultant lesion was crude, large, and difficult to reproduce precisely.[44] Some investigators noted that the active substance diffused from the site of administration, spreading to adjacent regions of the brain.[33] Furthermore, with alumina cream there is a long latency before the onset of seizures, ranging from 4 to 12 weeks, depending on the species.[15,48,49] The results are frequently inconsistent in a given species, with some animals apparently resistant to the effects of alumina cream while others die of status epilepticus.[47] Lastly, there appears to be a regional susceptibility to the epileptogenic properties of alumina cream: while the sensory motor cortex responds consistently, limbic structures require administration of destructively large quantities of the cream into both temporal lobes, and, even then, respond only inconsistently.[46,48] Clearly, this is a poor representation of limbic epilepsy in humans.

In spite of these drawbacks, the investigations with alumina cream demonstrated that limbic epilepsy can be produced by an exogenous chemical lesion, and revealed differential epileptogenicity of the cortical and limbic structures responding to the same agent. Also, they established that lesion size alone does not predict the degree of epileptogenicity.

Kainic acid

Kainic acid is an analog of glutamate, a powerful neuroexcitatory amino acid. In the late 1960s, kainic acid was found to have important excitatory effects on the cortical neurons of experimental animals.[50] Since then, many investigators have used kainic acid as an experimental epileptogenic agent and have described its effects on a variety of cortical and subcortical regions in different animals, most notably rats and mice.[33]

The primary site of action of kainic acid appears to be the hippocampus,[51] where it produces neuronal drop-out and sclerosis similar to that seen in the hippocampus of humans with temporal lobe epilepsy.[52] The associated electroclinical syndrome is similar in many ways to complex partial seizures in humans: focal epileptogenesis with complex, stereotyped symptoms and secondary generalization.[53] Similar events are observed after intraparenchymal,[51,52] intraventricular,[22] intravenous,[54] intraperitoneal, or subcutaneous administration of the agent.[55] This underscores the selective vulnerability of limbic structures to this agent, regardless of the site of administration. It is, thus, not surprising that the kainic acid model has emerged as an attractive and widely used model of temporal lobe epilepsy.[56–59]

The kainic acid model has been used widely to study neurotransmitter system homeostasis in limbic seizures. Changes in several neurotransmitter systems (including translation and transcription of specific receptors and enzymes, immunocytochemistry, and synaptic morphology) have been demonstrated and have been interpreted in light of various neurophysiopharmacologic theories.

The model has helped answer several questions about metabolic changes in and around the seizure focus. Studies with kainic acid refuted the hypothesis that neuronal damage in focal epilepsy is caused by metabolic demands increased beyond possible cerebral blood reserves. Instead, the concept of cell death by excitation, *excitotoxicity*, has emerged. This may explain neuronal damage not only in epilepsy but also in anoxia, ischemia, hypoglycemia, and other insults—all of which preferentially damage the same vulnerable cells in the hippocampus. The kainic acid model is also well suited to the investigation of potential pharmacologic and other treatment approaches in limbic epilepsy.

However, various investigators have observed that the effects of intraparenchymally administered kainic acid are not restricted to the site of the injection.[21,52,55,57,60] When ^{3}H-labeled kainate is administered interstitially, kainic acid molecules spread to a multitude of distant sites, including ipsilateral and contralateral regions of the brain.[61] The development of such distant lesions has been attributed to diffusion or to some form of specific neuronal transport of kainic acid to these areas.[33] Other distant lesions occur in areas without demonstrated presence of kainic acid. These have been attributed to the remote excitotoxic properties of the agent. Apparently, the genesis of some distant lesions can be aborted if the experimental animals are pretreated with diazepam, a potent anticonvulsant.[62]

Whatever the actual cause of such distant lesions, they do not occur to a detectable extent in human temporal lobe seizures, and they undoubtedly confuse the interpretation and analysis of results obtained

through kainic acid models, particularly in studies focusing on regional neurotransmitter alterations and metabolic changes in limbic epilepsy.

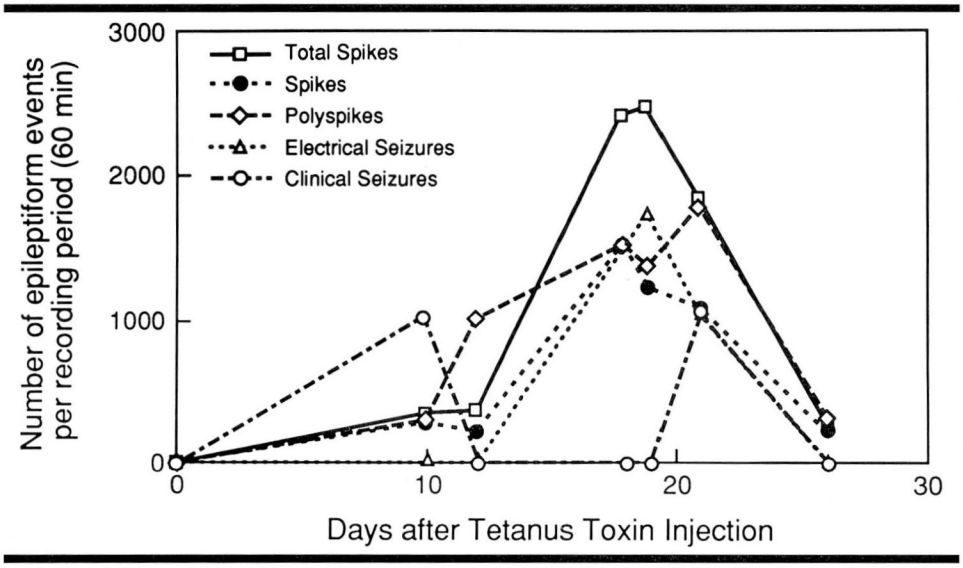

FIGURE 1. Focal limbic epileptogenesis following intrahippocampal tetanus toxin injection. Serial recordings of epileptiform activity and clinical seizures using a depth electrode in a single animal.

Tetanus toxin

Tetanus toxin is a protein produced by the anaerobic bacterium *Clostridium tetani*. It consists of two polypeptide chains linked by two disulfide bonds and has a molecular weight of 150,000.[63] A potent neurotoxin, it binds to gangliosides and consequently undergoes retrograde axonal transport to neural synapses where it blocks the presynaptic release of GABA and glycine, both of which are powerful inhibitory neurotransmitters.[64] It is still not clear what happens to the toxin after it reaches the central nervous system. Some of the toxin appears to leave the cell body in which it arrives, and this may allow it to exert postsynaptic action as well. It is still debatable whether there is any further uptake or transport of the toxin within the central nervous system.[63]

Tetanus toxin was first used to induce chronic epileptogenic lesions in dogs in 1962.[65] When tetanus toxin is administered intraparenchymally (in small doses, many times lower than that required to produce tetanus) into the brain of experimental animals, there is a relatively short latency before the clinical and electroencephalographic onset of seizures. This latency ranges from a few hours to several weeks.[66] Tetanus toxin induces foci that may remain active for a long time and then undergo spontaneous extinction. Mellanby and associates[67] induced foci in rat hippocampi that remained active for several weeks. The lesion produced by tetanus toxin is discrete, consisting only of a small area of necrosis and reactive gliosis limited to the site of injection.[33] There is no detectable diffusion of the toxin outside the area of intraparenchymal administration, and there are no diffuse areas of neuronal damage.[67]

Mellanby and associates have characterized a tetanus toxin model of limbic epilepsy in the rat, describing its time course and clinical, electrical, and pathologic features. Apparently, the animal passes through three stages of focus development (*Figure 1*). The first stage is that of focus maturation, in which there is a gradual buildup of focal epileptiform electroencephalographic activity but no overt clinical seizures. The second stage is that of focus expression, where the animal exhibits stereotyped clinical seizures resembling psychomotor seizures in humans and kindled limbic seizures in rats (see "Kindling," above). The last stage is that of focus extinction, when both clinical and electrical activity gradually disappear.[67]

Although experience with this model is still relatively limited, its advantages include the lack of distant neuronal damage and diffusion of the toxin. It is therefore a more precise model of focal limbic epilepsy. The electroclinical syndrome is stereotyped and typical of clinical and other experimental complex partial seizures. The model allows investigation of regional neurochemical and metabolic phenomena accompanying limbic epilepsy without potentially confusing distant pathologic effects. Furthermore, the model allows the investigation of epileptogenesis per se (during the phase between toxin injection and the appearance of clinically overt seizures), epileptic expression and perpetuation (during the phase of overt seizures), and recovery from seizures (during extinction of the seizure focus). The tetanus toxin model can be used to address

many of the same investigative questions as the kainic acid model.

PATHOLOGY OF LIMBIC EPILEPSY: CURRENT CONCEPTS

Experimental models of limbic epilepsy have helped clarify several basic mechanisms involved in limbic epilepsy. Several hypotheses related to these mechanisms have been proposed, allowing the formulation of a general pathophysiologic scheme. These concepts are essential to current and future clinical interventions for this disorder.

Genetic and developmental vulnerabilities

Although no specific genetic abnormality has been incriminated as a cause of limbic epilepsy, it is now generally accepted that there is a genetic basis for susceptibility to seizures.[68,69] That there are susceptible strains of animals supports this hypothesis and has made studies on genetic markers and mechanisms of vulnerability possible.

The kindling model has demonstrated that susceptibility to limbic epileptogenesis varies among species and developmental stages in the same species. Generally, limbic structures are less easily kindled in higher animals. Higher animals, including primates, are also more vulnerable to kindling at critical stages of neuronal development.[70,71] This may explain human susceptibility to hippocampal sclerosis and limbic epilepsy as a result of neural insults early in life. Much remains to be learned about factors predisposing to such vulnerability and about ways of modifying it.

Neuronal abnormalities and the neuronal milieu

Some form of abnormality (structural lesion or otherwise) is presumed to exist in the brain, particularly in the mesial temporal lobe region, in cases of limbic epilepsy. The "lesion" renders individual neurons more excitable or reorganizes neuronal circuitry in a manner that enhances synchronization. The alumina cream and kainic acid models have demonstrated that such a "lesion" disturbs ionic channels and increases excitability of individual neurons. Also, synaptic terminals are destroyed, thus attenuating inhibitory influences, or neuronal circuitry sprouts and reorganizes, resulting in excitatory phenomena. In several animal models of limbic epilepsy, changes in neurotransmitter homeostasis have been demonstrated: many neurotransmitters have been implicated, including GABA, somatostatin, opioid peptides, and others. Such disturbances in neurotransmitter profiles have been associated with development of intermittent, synchronized burst discharges within certain neuronal aggregates. As evidenced by the kindling model, such repeated burst discharges may then induce further reorganization of neuronal connections, thereby perpetuating epileptic activity.[72]

Secondary epileptogenesis

Experiments using the kindling model and the kainic acid model have emphasized the influence of epilepsy in one region of the brain, on the surrounding brain, and on more distant structures. In certain instances, this may result in novel epileptogenicity in adjacent and distant structures, reflecting an "expansion" of the epileptic focus or the genesis of distant secondary epileptic foci.

Role of inhibition

Although loss of inhibition has been thought to be essential to the synchronization of neuronal activity that characterizes epileptiform discharges, there is increasing evidence of a "paradoxical" phenomenon of enhanced inhibition in brain regions surrounding the epileptic focus. Neurons in the epileptic focus appear to have a low firing rate. Epileptogenic neurons are thus *hypersynchronized* but not *hyperactive*. Strong inhibitory influences may help maintain the interictal state and may prevent ictal onset. Studies using the kindling and kainic acid models have shown that interictal inhibition may be partly mediated by GABA and opioid peptides. Transition from the interictal to the ictal state and the spread of epileptic discharges to adjacent brain tissue suggest the intermittent breakdown of such inhibitory influences.[72]

Such inhibitory activity within and around the epileptic focus is reflected in interictal hypometabolism and decreased cerebral blood flow, which can be detected with functional neuroimaging techniques, including positron emission tomography, single-photon emission tomography, and magnetic resonance imaging. These techniques have allowed us to localize epileptic foci in humans. However, much of the relationship between structural lesions and such inhibition remains to be elucidated. It is also not known whether the inhibition is the result of neuronal dysfunction from epilepsy or is a homeostatic response of normal brain in an effort to contain seizure activity.

FUTURE DIRECTIONS AND CLINICAL RELEVANCE

Animal models of limbic epilepsy have helped us

formulate theories of how epileptogenic foci and the interictal state develop and the transition of the latter to the ictal state. However, many questions remain unanswered and must be tackled through further research. *Figure 2* summarizes several general concepts of focal epileptogenicity. There are effects of a structural lesion on surrounding brain promoting epileptogenesis, and there are reciprocal effects of epileptogenesis causing a structural lesion (excitotoxicity). This may promote further epileptogenicity or the appearance of new lesions. The reaction of the brain to focal epileptic activity needs to be clarified further. Is the inhibitory activity surrounding an epileptic focus actually a healthy phenomenon designed to contain seizure activity? If so, which specific neurotransmitter systems are responsible for mediating it? Alternatively, is this inhibition a manifestation of progressive brain dysfunction caused by continuing seizure activity? Answers to these questions will undoubtedly expose new horizons in the diagnosis and treatment of complex partial seizures.

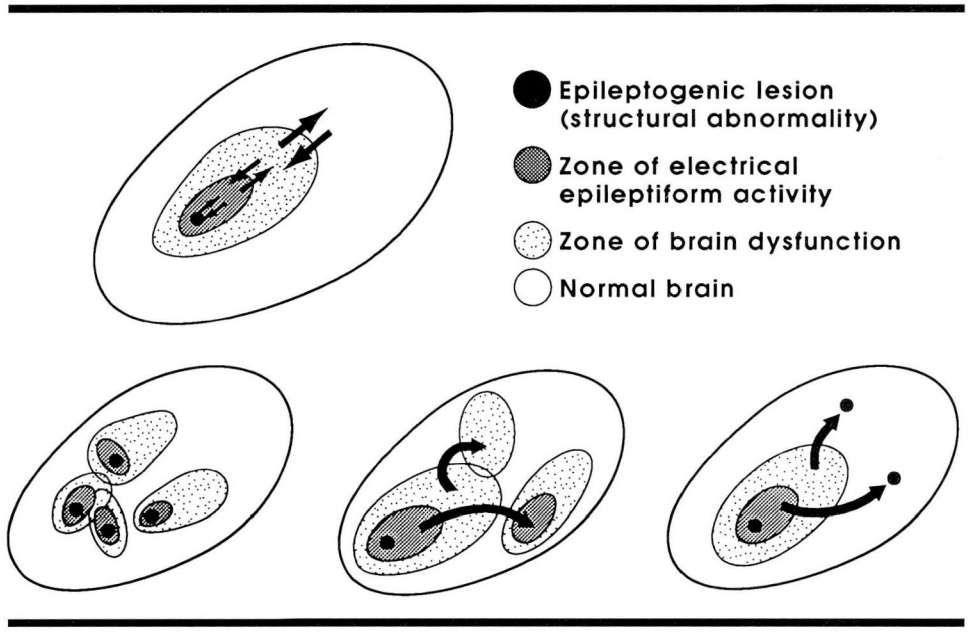

FIGURE 2. Interactions that may be relevant in focal limbic epilepsy. The relationships of lesions to surrounding epileptogenic brain and to normal brain are not well understood (top). It is also not known how multiple lesions interact within overlapping zones of epileptogenesis (lower left). Related and poorly understood phenomena include seizure-induced neuronal damage, or excitotoxicity (lower center) and the contribution of damaged areas (secondary lesions) to further epileptogenesis (lower right).

The field of future research on experimental animal models of limbic epilepsy is immense. Many of the basic pathophysiologic mechanisms involved in limbic epilepsy are still unclear. The role of neurotransmitter systems in various brain regions during development of an epileptogenic focus and how this development is expressed clinically need further elucidation. Somatostatin has been proposed as a causative agent or as an indicator of maturation of an epileptic focus.[39] The GABA system, a potent natural inhibitory system, has been implied in the strong inhibitory influences during the interictal state.[28,72] Opioid peptides have been paradoxically related to both epileptogenicity and to anticonvulsant activity through various receptor subtypes.[73] The biochemistry of these receptors and their effects on epileptic neurons will further clarify these mechanisms and may provide new therapies to prevent maturation and expression of seizures.

Better understanding of the various neurotransmitter systems will have a profound impact on the prevention and treatment of complex partial seizures. When their sites and mechanisms of action are known, we may be able to suppress or promote their activity with drugs. Already, clinical trials on several such drugs are underway.

Vigabatrin (gamma-vinyl GABA) is a drug that increases synaptic GABA concentrations by inhibiting its catabolic enzyme, GABA transaminase. Animal studies have demonstrated a broad spectrum of antiepileptic activity for this drug.[74] Clinical trials are in progress on this agent and on another GABA transaminase inhibitor, milacemide (2-n-pentylaminoacetamide).[75] Mercaptamine, a somatostatin-depleting drug, has been shown to suppress seizures in amygdala-kindled rats but has not been tried in humans.[39] The future promises development of numerous antiepileptic drugs that may be far more effective than the currently used anticonvulsants, with focused activity on target structures and, perhaps, with fewer side effects.

Future animal research on the role of neurotransmit-

ters in epilepsy may open new horizons in the field of brain grafting. It is not unrealistic to foresee brain grafting procedures aimed at increasing or decreasing the concentration of certain neurotransmitters at specific regions of the brain to control seizures.

Research on neurotransmitter activity in epilepsy may have other benefits in diagnosis. In medically intractable complex partial seizures, positron emission tomography has revealed interictal zones of hypometabolism corresponding to the general areas surrounding zones of ictal onset. However, such hypometabolic zones are diffuse and often extend well beyond the limits of the "electric" focus. Thus far, the role of such functional imaging in the preoperative evaluation of epileptic patients has been corroborative, but nonspecific. If the exact role of the various neurotransmitter systems in epilepsy were known, it would be possible to precisely outline the area of the brain responsible for the seizure using ultraspecific positron emission tomography in conjunction with specific receptor ligands.

The future also promises more intensive research on the genetic basis of epilepsy. Further research should bring to light genetic abnormalities associated with vulnerability to different types of epilepsy, including limbic seizures. In the coming era of genetic engineering, it may be possible to diagnose such genetic abnormalities and develop strategies at the molecular and genetic levels to preempt or contain epileptogenic insults before the epileptic focus fully matures and expresses seizures.

Obviously many questions cannot be answered through clinical research alone or by examining human tissue. By the time limbic epilepsy is diagnosed and comes to medical and surgical treatment, many of the pathophysiologic processes are already advanced. Manipulating these processes in a controlled setting and testing specific hypotheses may therefore be prohibitive in the clinical setting, for practical and ethical reasons. Animal models of limbic epilepsy can provide a suitable milieu for answering questions that can be investigated only in complex functional neuronal systems in vivo. We have learned much that is clinically relevant from these animal models and will be able to learn much more with future investigations.

Yet, such experimental models have limitations. The controlled laboratory environment hardly represents the complex social and behavioral environment of human epilepsy. Phylogenetic differences may prevent applying experimental discoveries to humans. However, experimental models are an important bridge between molecular investigations and clinical studies. Without this bridge, the rapidly expanding knowledge of basic neuroscience will never be applied clinically. More refined models and improved experimental techniques are needed to tackle the many remaining questions, but the resulting knowledge will translate into innovative diagnostic and therapeutic strategies.

REFERENCES

1. National Institutes of Health. Draft for consensus development conference statement on surgery for epilepsy. Neurology, in press.
2. Hauser WA, Kurland LT. The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. Epilepsia 1975; **16**:1–66.
3. Gastaut H, Gastaut JL, Goncalves-e-Silva GE, Fernandez Sanchez GR. Relative frequency of different types of epilepsy: a study employing the classification of the international league against epilepsy. Epilepsia 1975; **16**:457–461.
4. Schmidt D, Einicke I, Haenel F. The influence of seizure type on the efficacy of plasma concentration of phenytoin, phenobarbital and carbamazapine. Arch Neurol 1986; **43**:263–265.
5. Suzuki J, Nakamoto Y. Seizure patterns and electroencephalograms of EL mouse. Electroencephalogr Clin Neurophysiol 1977; **43**:299–311.
6. Mutani R. Cobalt experimental hippocampal epilepsy. Epilepsia 1967; **8**:223–240.
7. Blum B, Liban E. Experimental baso-temporal epilepsy in the cat. Discrete epileptogenic lesions produced in the hippocampus or amygdaloid by tungstic acid. Neurology 1960; **10**:546–554.
8. Matsumoto H, Aijmone-Marsan C. Cortical cellular phenomena in experimental epilepsy: interictal manifestations. Neurology 1964; **9**:286–304.
9. Kopeloff LM, Barrera SE, Kopeloff N. Recurrent convulsive seizures in animals produced by immunologic and chemical means. Am J Psychiatry 1942; **98**:881–902.
10. Pei Y, Zhao D, Huang T, et al. Zinc-induced seizures: a new experimental model of epilepsy. Epilepsia 1983; **24**:169–176.
11. Morrell F, Bradley W, Ptashne M. Effects of drugs on discharge characteristics of chronic epileptogenic lesions. Neurology 1959; **9**:492–498.
12. Wada JA, Mizoguchi T, Komai S. Kindling epileptogenesis in orbital and mesial frontal cortical areas of subhuman primates. Epilepsia 1985; **26**:472–479.
13. Wauquier A, Melis W, Desmedt LKC, Sadowski B. Self stimulation in dogs: behavioral effects of anterior basal forebrain, amygdala and lateral hypothalamus implantations. In: Wauquier A, Rolls ET eds. Brain Stimulation Record. Amsterdam: North-Holland, 1976: 427–430.
14. Wauquier A, Ashton D, Melis W. Behavioral analysis of amygdaloid kindling in beagle dogs and the effects of clonazepam, phenobarbital, diphenylhydantoin, and flunarizine on seizure manifestation. Exp Neurol 1979; **64**:579–586.
15. Gastaut H, Naquet R, Meter A, et al. Clinical, electroencephalographic and anatomo-pathological study of "psychomotor" epilepsy induced in the cat by injection of alumina cream. In: Baldin M, Bailey P, eds. Temporal Lobe Epilepsy. Springfield, IL: Charles Thomas Publ., 1958: 240–242.
16. Feria-Velasco A, Olivares N, Rivas F, et al. Alumina cream-induced focal motor epilepsy in cats. IV. Thickness and cellularity of layers in the perilesional motor cortex. Arch Neurol 1980; **37**:287–290.
17. Beaumanoir A, Naquet R, Vigouroux R. Temporal lobe epilepsy: experimental reproduction. In: Broughton RJ, ed. Henri Gastaut and the Marseilles School's Contribution to the Neurosciences (EEG

Suppl Number 35) Amsterdam: Elsevier Biomedical Press, 1982: 159–170.
18. Ben-Ari Y, Lagowska J, Tremblay E, et al. A new model of focal status epilepticus: intra-amygdaloid application of kainic acid elicits repetitive secondary generalized convulsive seizures. Brain Res 1979; **163:**176–179.
19. Ben-Ari Y, Tremblay E, Ottersen OP, et al. The role of epileptic activity in hippocampal and "remote" cerebral lesions induced by kainic acid. Brain Res 1980; **191:**79–97.
20. Olney JW, Fuller Ta, de Gubareff T. Kainate-like neurotoxicity of folates. Nature 1981; **292:**165–167.
21. Tremblay E, Ottersen OP, Rovira C, et al. Intra-amygdaloid injections of kainic acid: regional metabolic changes and their relation to the pathological alterations. Neuroscience 1983; **8:**299–315.
22. Nadler JV, Perry BW, Cotman CW. Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. Nature 1978; **261:**676–677.
23. Olney JW, Rhee V, Lan Ho O. Kainic acid: a powerful neurotoxic analogue of glutamate. Brain Res 1974; **77:**507–512.
24. Morrell F, Tsuru N, Hoeppner TJ, Morgan D, Harrison WH. Secondary epileptogenesis in frog forebrain: effect of inhibition of protein synthesis. In: Wada JA, ed. Kindling. New York: Raven Press, 1976: 41–60.
25. Rial RV, Gonzalez J. The effect of diphenylhydantoin in the prevention of the threshold descents in the electroshock on the reptilian telencephalon. In: Majkowski J, ed. Post-traumatic Epilepsy. Warsaw: Polish Chapter of the ILAE, 1977: 203–208.
26. Sugaya E, Ishige A, Sekiguchi K, et al. Pentylenetetrazol-induced convulsions and the effect of anticonvulsants in mutant inbred strain EL mice. Epilepsia 1986; **27:**354–358.
27. Yamagami S, Onishi H, Ohno K, Mori K, Kawakita Y. Alteration of RNA synthesis in vitro in intact cerebral cortex nuclei induced by convulsions in seizure-susceptible EL mice. Exp Neurol 1987; **95:**167–177.
28. King JT, LaMotte CC. VIP-, 55, and GABA-like immunoreactivity in the midhippocampal region of EL (epileptic) and C57BL/6 mice. Brain Res 1988; **475:**192–197.
29. Delgado JMR, Sevillano M. Evolution of repeated hippocampal seizures in the cat. Electroencephalogr Clin Neurophysiol 1961; **13:**722–733.
30. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 1969; **25:**295–330.
31. McIntyre DC, Racine RJ. Kindling mechanisms: current progress on an experimental epilepsy model. Prog Neurobiol 1986; **27:**1–12.
32. SchwartzKroin PA, Wyler AR. Mechanisms underlying epileptiform burst discharge. Ann Neurol 1980; **7:**95–107.
33. Louis ED, Williamson PD, Darcey TM. Experimental models of chronic focal epilepsy: a critical review of four models. Yale J Biol Med 1987; **60:**255–272.
34. Wada JA, Osawa J, Mizoguchi T. Recurrent spontaneous seizure state induced by prefrontal kindling in sengalese baboons, *Papio papio*. In: Wada JA, ed. Kindling. New York: Raven Press, 1976: 73–202.
35. Racine RJ. Modification of seizure activity by electrical stimulation: I. After-discharge threshold. Electroencephalogr Clin Neurophysiol 1972; **32:**269–279.
36. Leech CK, McIntyre DC. Kindling rates in inbred mice: an analog to learning? Behav Biol 1976; **16:**439–452.
37. McNamara JO. Kindling: an animal model of complex partial epilepsy. Ann Neurol 1984; **16 (suppl):**572–576.
38. Gean PW, Gallagher PS, Anderson AC. Spontaneous epileptiform activity and alteration of GABA- and of NMDA-mediated neurotransmission in amygdala neurons kindled in vivo. Brain Res 1989; **494:**177–181.
39. Pitkanen A, Hyttinen JM, Riekkinen PJ. Effect of cysteamine on somatostatin-like immunoreactivity in the anygdala-kindled rat brain. Neuropeptides 1989; **14:**11–16.
40. McGinty JF, Kanamatsu T, Obie J, et al. Amygdaloid kindling increases enkephalin-like immunoreactivity but decreases dynorphin-A-like immunoreactivity in rat hippocampus. Neurosci Letters 1986; **71:**31–36.
41. Engel J Jr., Cahan L. Potential relevance of kindling to human partial epilepsy. In: Wada J, ed. Kindling 3. New York: Raven Press, 1986: 37–51.
42. Burnham W. Primary and "transfer" seizure development in the kindled rat. In: Wada JA, ed. Kindling. New York: Raven Press, 1976: 61–83.
43. Westrum LE, White LE, Ward AA. Morphology of the experimental epileptic focus. J Neurosurg 1964; **21:**1033–1046.
44. Harris AB. Ultrastructure and histochemistry of alumina in the cortex. Exp Neurol 1973; **38:**33–63.
45. Velasco M, Velasco F, Marquez I, et al. Alumina cream-induced focal motor epilepsy in cats. VI. Excision of the perilesional cortex. Electroencephalogr Clin Neurophysiol 1985; **60:**548–557.
46. Soper HV, Strain GM, Babb TL, et al. Chronic alumina temporal lobe seizures in monkeys. Exp Neurol 1978; **62:**99–121.
47. Mayanagi Y. Alumina cream-induced temporal lobe epilepsy in the monkey as an experimental model. Folia Psychiatr Neurol Jap 1979; **33:**457–462.
48. Stamn JS, Pribram KH. Effects of epileptogenic lesions of inferotemporal cortex on learning and retention in monkeys. J Comp Physiol Psychol 1961; **6:**614–618.
49. Mayanagi Y. Experimental studies on the pathogenesis of temporal lobe epilepsy. Folia Psychiatr Neurol Jap 1976; **30:**415–424.
50. Shinozaki H, Konishi S. Actions of several antihelminthics and insecticides on rat cortical neurones. Brain Res 1970; **24:**368–371.
51. Ben-Ari Y. Limbic seizures and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 1985; **14:**374–403.
52. Pisa M, Sanberg PR, Corcoran ME. et al. Spontaneously recurrent seizures after intracerebral injections of kainic acid in rat: a possible model of human temporal lobe epilepsy. Brain Res 1980; **200:**482–487.
53. Schwartz R, Coyle JT. Neurochemical sequelae of kainate injections in corpus striatum and substantia nigra of the rat. Life Sci 1977; **20:**431–436.
54. Tremblay E, Nitecka L, Berger ML, et al. Maturation of kainic acid seizure-brain damage syndrome in the rat. I. Clinical electrographic and metabolic observations. Neuroscience 1984; **13:**1051–1072.
55. Sperk G, Lassmann H, Baran H, et al. Kainic acid induced seizures: neurochemical and histopathological changes. Neuroscience 1983; **10:**1301–1315.
56. Nitrecka L, Tremblay E, Charton G, et al. Maturation of kainic acid seizure-brain damage syndrome in the rat. II. Histopathological sequelae. Neuroscience 1984; **13:**1073–1094.
57. Nadler JV. Minireview: kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci 1981; **29:**2031–2042.
58. Cavalheiro EA, Riche DA, Le Gal La Salle G. Long-term effects of intrahippocampal kainic acid injection in rats: a method for inducing spontaneous recurrent seizures. Electroenceph Clin Neurophysiol 1982; **53:**581–589.
59. French Ed, Aldinio C, Schwarcz R. Intrahippocampal kainic acid, seizures and local neuronal degeneration: relationships assessed in unanesthetized rats. Neuroscience 1982; **7:**2525–2536.
60. Menini C, Meldrum BS, Riche D, et al. Sustained limbic seizures induced by intraamygdaloid kainic acid in the baboon: symptomatology and neuropathological consequences. Ann Neurol 1980; **8:**501–509.
61. Zaczek R, Simonton S, Coyle JT. Local and distant neuronal degeneration following intrastriatal injection of kainic acid. J Neuropathol Exp Neurol 1980; **39:**245–264.
62. Ben-Ari Y, Tremblay E, Ottersen OP, et al. Evidence suggesting secondary epileptogenic lesions after kainic acid: pretreatment with diazepam reduces distant but not local brain damage. Brain Res 1979; **165:**362–365.
63. Mellanby J, Green J. How does tetanus toxin act? Neuroscience 1981; **3:**281–300.
64. Van Heyningen S. Tetanus Toxin. Pharmacol Ther 1980; **11:**141–157.
65. Carrea R, Lanari A. Chronic effect of tetanus toxin applied locally

to the cerebral cortex of the dog. Science 1962; **137:**342–343.
66. McGreer PL, McGreer EG, Campbell JJR. Rotary effects of intracerebral tetanus toxin injections. Exp Neurol 1980; **67:**363–367.
67. Mellanby J, George G, Robinson A, Thompson P. Epileptiform syndrome in rats produced by injecting tetanus toxin into the hippocampus. J Neurol Neurosurg Psychiatry 1977; **40:**404–414.
68. Andermann E. Multifactorial inheritance of generalized and focal epilepsy. In: Anderson VE, Hansen WA, Penry JK, Sing CF, eds. Genetic Basis of the Epilepsies. New York: Raven Press, 1982: 355–374.
69. Newmark ME, Penry JK. Genetics of Epilepsy: A Review. New York: Raven Press, 1980: 120–122.
70. De Toledo-Morrel L, Morrell F, Fleming S. Age-dependent deficits in spatial memory are related to impaired hippocampal kindling. Behav Neurosci 1984; **98:**902–907.
71. Moshe SL, Albala BJ, Ackermann RF, et al. Increased seizure susceptibility of the immature brain. Developmental Brain Research 1983; **7:**81–85.
72. Engel J. Jr. Seizures and Epilepsy. Philadelphia: F.A. Davis Company, 1989: 71–111.
73. Tortella FC. Endogenous opioid peptides and epilepsy: quieting the seizing brain. Trends Pharmacol Sci 1988; **9:**366–372.
74. Schechter PJ. Vigabatrin. In: Medlrum BS, Porter RJ, eds. Current Problems in Epilepsy Vol. 4, New Anticonvulsants. London: John Libbey, 1986: 265–275.
75. Roba J, Cavalier R, Cordi A, et al. Milacemide. In: Meldrum BS, Porter RJ, eds. Current Problems in Epilepsy Vol 4, New Anticonvulsant Drugs. London: John Libbey, 1986: 179–190