34. F. Songling *et al.*, in *Proceedings of the Third Marcel Grossmann Meeting on General Relativity,* H. Ning, Ed. (North-Holland, Amsterdam, 1983), pp. 707–718.
35. W. Hamilton, D. Darling, J. Kadlec, D. DeWitt, *ibid.*, pp. 1175–1178.
36. V. B. Braginsky, V. P. Mitrofanov, V. I. Panov, *Systems with Small Dissipation* (Univ. of Chicago Press, Chicago, 1985).
37. W. Davis *et al.*, in *Proceedings of the Third Marcel Grossmann Meeting on General Relativity,* H. Ning, Ed. (North-Holland, Amsterdam, 1983), pp. 1433–1436.
38. M. Fujimoto, *ibid.*, pp. 1447–1450.
39. D. Blair *et al.*, *ibid.*, pp. 719–738.
40. We gratefully acknowledge the important contributions of our colleagues at Stanford and elsewhere to the work described here. In particular we thank W. M. Fairbank, M. S. McAshan, and T. Stevenson for valuable discussions. The experimental effort at Stanford University is supported by National Science Foundation grants PHY85-13525, PHY85-15856, and INT84-19953.

# Cellular Mechanisms of Epilepsy: A Status Report

MARC A. DICHTER AND G. F. AYALA

**The cellular phenomena underlying focal epilepsy are currently understood in the context of contemporary concepts of cellular and synaptic function. Interictal discharges appear to be due to a combination of synaptic events and intrinsic currents, the exact proportion of which in any given neuron may vary according to the anatomic and functional substrate involved in the epileptic discharge and the epileptogenic agent used in a given model. The transition to seizure appears to be due to simultaneous increments in excitatory influences and decrements in inhibitory processes—both related to frequency-dependent neuronal events. A variety of specific hypotheses have been proposed to account for the increased excitability that occurs during epileptiform activity. Although each of the proposed mechanisms is likely to contribute significantly to the epileptic process, no single hypothesis provides an exclusive unifying framework within which all kinds of focal epilepsy can be understood. The spread of epileptic activity throughout the brain, the development of primary generalized epilepsy, the existence of "gating" mechanisms in specific anatomic locations, and the extrapolation of hypotheses derived from simple models of focal epilepsy to explain more complex forms of human epilepsy, all are not yet fully understood.**

THE EPILEPSIES ARE A FAMILY OF NEUROLOGICAL DISORders that have in common a transient, recurrent, self-sustained interruption of normal brain functions and a simultaneous hypersynchronous activation of a large population of neurons in one focal area or generally throughout the brain. Attempts to understand epilepsy have always been based on prevailing concepts of central nervous system (CNS) physiology, and over many years, advances in understanding CNS function have provided progressively more sophisticated hypotheses to explain epileptic activity. In this article we examine the hypotheses that have been proposed and discuss the data supporting them, distinguishing evidence derived from studies of epileptic models from arguments based on extrapolations from normal cellular physiology. This article also indicates areas in which important information is still lacking and attempts to relate current hypotheses about cellular mechanisms of focal epilepsy to the larger context of a network analysis of epilepsy in other kinds of models and, where possible, the phenomenology of human epilepsy.

One of the most influential early conceptual models of epileptic mechanisms, based on clinical and electroencephalographic (EEG) observations, postulated that epilepsy existed in two broad forms—focal or partial epilepsy due to a discrete cortical abnormality and generalized or centrencephalic epilepsy due to an abnormality in deep subcortical gray matter. It was suggested that the paroxysmal activity of the latter type invaded the forebrain through neuronal structures with diffuse projections (*1*). This model implied (i) that the generalization of a seizure initiated at a cortical focus would occur when the cortical paroxysmal activity spread to these deep structures and (ii) that the primary generalized form of epilepsy was also the expression of a "focal" activity localized in deep structures. Many different kinds of experimental models that reproduced the two hallmarks of these forms of human epilepsy, clinical convulsions and EEG "spikes," were used to study the roles of different brain structures and anatomic pathways in the development and spread of epileptiform activity.

Modern research into the cellular mechanisms of epilepsy began 30 years ago when extracellular and intracellular recordings from the vertebrate CNS became possible and, more importantly, the mechanisms underlying neuronal excitability and synaptic functions began to be unraveled (*2*). Research was concentrated on models of cortical focal epilepsy, both acute and chronic, as these provided scientists with a clear target for their microelectrodes and anatomic probes, and these models appeared to represent several components of the human condition. The goals were to understand the altered physiology of individual neurons or synaptic function that underlies the regional epileptiform activity, with the hope of developing pharmacological tools that could be used to suppress the abnormal activity. By 1970, the phenomena that occur in neurons within these experimental foci during cortical interictal discharges and seizures

M. A. Dichter is director of the Comprehensive Epilepsy Center, Graduate Hospital, and University of Pennsylvania, Philadelphia, PA 19146. G. F. Ayala holds the chair of Neurophysiology, University of Rome, "Tor Vergata," Rome, Italy, and is also with the Department of Neurology, Medical College of Ohio, Toledo, OH 43606.

had been described and hypotheses were developed to explain them, based on contemporary knowledge of neuronal and synaptic function (*3–11*). These hypotheses defined the direction of much of epilepsy research over the last 15 years, during which new techniques in cellular neurophysiology, neuropharmacology, and biophysics provided a much more detailed view of intrinsic membrane events and synaptic mechanisms. As a consequence of this new information, the original hypotheses about the mechanisms underlying interictal discharges and seizures have been progressively expanded and refined. However, the study of epilepsy has also become more reductionist, and, although elegant analyses are being performed on a few epilepsy models, questions continue to arise about the relevance of these experimental models to human epilepsy and about mechanisms underlying other forms of epilepsy, such as generalized epilepsy, about which much less is known.

Much effort, over the past two decades, has been spent on developing a single "unified" hypothesis about the cellular mechanisms of epilepsy. As new mechanisms of normal membrane or synaptic function have been uncovered, they have been used as keys to understanding the epilepsy puzzle, creating, over the years, a pattern of "trendy" theories. For example, hypotheses that emphasized the "epileptic neuron," the synaptic aggregate, γ-aminobutyric acid (GABA), endogenous bursting neurons, extracellular potassium or calcium, and *N*-methyl-D-aspartate (NMDA) receptors have been proposed as the key to understanding epilepsy. This article will attempt to place each of these elements into the context of an integrated view of mechanisms underlying epileptiform phenomena as they are seen from the perspective of the experimental and clinical neurophysiologist.

## Experimental Focal Epilepsy in Vivo

The EEG hallmarks of focal epilepsy both in animal models and in human epilepsy are the ictal, or seizure, discharge and the interictal spike discharge (ID). The EEG spike most often represents an electrophysiological marker for a hyperexcitable area of cortex and arises in or near an area with a high epileptogenic potential. As such, it has been considered the earliest and simplest electrical manifestation of the epileptic process and has been the target of extensive investigations. In some forms of epilepsy, seizure discharges can be seen to originate electrically and anatomically from the site of spike discharges, and the transition between spikes and seizures has been analyzed in these simple models. In other forms of epilepsy, however, the exact relation between spike discharge and the onset and localization of seizure discharges has been more difficult to determine. We will first review our current understanding of the simple focal seizure models, where ID and seizures seem most closely related, and then discuss our current understanding of other forms of seizure phenomena.

In acute focal epilepsy, during the ID, thousands of neurons in the focus synchronously undergo an unusually large depolarization (the depolarizing shift or DS), superimposed on which is a burst of action potentials. The DS is followed by a hyperpolarizing potential (the post-DS HP) and neuronal inhibition (*3–11*). In areas surrounding the focus, many neurons are inhibited during the ID (*7, 12*). In distant projection areas, neurons can be excited briefly but more often are inhibited during the ID, according to their synaptic interactions (*13*). Axons that end within the focus generate action potentials, which can "backfire" and propagate antidromically (*14*). In addition, during the ID, at the site of the focus, extracellular levels of $K^+$ increase and levels of $Ca^{2+}$ decrease, presumably because of exit of $K^+$ from and entry of $Ca^{2+}$ into neuronal processes during the intense neuronal activity (*15–17*).

When seizures develop, at least in the acute focus, the neurons show a characteristic sequence of events: the post-DS HP becomes smaller, gradually disappears, and is replaced by a depolarization, on top of which are smaller depolarizing waves that resemble small DSs (*4, 8, 10*). This series of events occurs synchronously in the population of neurons within the focus, and the EEG develops after discharges (ADs) after several successive IDs. The ADs become longer with each ID and then progress into a seizure. Meanwhile, near and distant areas of brain are brought into the seizure process, and the abnormal activity spreads. During this process, levels of extracellular $K^+$ continue to increase until they reach a steady-state level well above normal, and levels of extracellular $Ca^{2+}$ continue to decrease. Finally the seizure subsides, and the neuronal membrane hyperpolarizes well beyond control level. It is not known whether this orderly progression from IDs to seizures occurs in the same way in chronic epileptic foci or in many forms of human epilepsy. Depth electroencephalography from humans with focal epilepsy has demonstrated multiple patterns during the transition to seizure, only some of which resemble that seen in acute experimental focal epilepsy (*18*). Whether these observations indicate that the mechanisms underlying the transition in chronic human foci are different from those of the simple acute model is not yet clear.

Each of the phenomena discussed above needs to be analyzed to understand the mechanisms that generate the focal epileptiform paroxysm, limit the temporal and spatial spread of IDs, and break down when the transition to seizure develops.

## Cellular Mechanisms Responsible for Epileptic Events

Early investigations of epileptic mechanisms attempted to determine whether the primary abnormality resided in altered intrinsic neuronal properties or in the aggregate properties of neuronal networks. It is now clear that a variety of inciting agents used to generate experimental models of epilepsy have apparently unrelated primary mechanisms of action (Table 1), and yet they can all produce epileptic activity that is characterized by the stereotyped cellular phenomena that we have described; that is, the DS HP sequence and surround inhibition. Thus, alterations in intrinsic membrane properties or in synaptic function can produce focal epilepsy. In more complex models of focal epilepsy, such as the chronic alumina cream model, both intrinsic membrane properties and synaptic function appear to be altered (*19*). Normal neuronal architecture is disrupted because many neurons are lost [especially the inhibitory interneurons (*20*)]. Other neurons show bent or shortened dendrites and loss of dendritic spines. The neurons in these foci have abnormal firing patterns, but neither the mechanism of the abnormal firing nor the relative importance of the intrinsic or synaptic abnormalities in the development of the epileptic events is well understood. However, in this model, as in the acute focal model, the neurons within the area from which IDs are generated undergo a DS HP sequence that appears indistinguishable from the DS seen in acute foci (*21*).

In both acute and chronic foci, however, regardless of the anatomical and functional changes of the neurons within the focus itself, or the mechanism of action of the epileptogenic agent, the majority of neurons that become involved as a seizure spreads from the focus are "normal" (*22*). This emphasizes that two sets of epileptic mechanisms must be involved: one that underlies the development of epilepsy in a focal area physically exposed to the epileptogenic agent, and one that underlies the conversion into epileptic activity of normal CNS activity remote from the focus, in the absence of exogenously applied drugs or chronic lesions.

## The Depolarizing Shift

Theories about the origin of the DS that underlies the ID are based on evidence derived from studies of epileptic models and from extrapolations from studies of normal cellular physiology of cortex. The evidence from epileptic models where the synaptic mechanisms are not completely blocked indicates that the DS is generated by an excitatory synaptic current that may be amplified by the activation of intrinsic voltage-dependent membrane currents (*7–11, 23, 24*). The relative proportion of each of these components may vary in different neurons, but it appears that in the majority of both neocortical and archicortical neurons, synaptic currents are the predominant mechanism.

In order to generate the large excitatory postsynaptic potential (EPSP) underlying the DS, the normal EPSP, which is small and often masked by powerful inhibition, must be amplified. At least five mechanisms exist in the normal CNS that could cause enhancement of EPSPs in epileptic foci: (i) withdrawal of inhibition, (ii) frequency potentiation of EPSPs (*25*), (iii) changes in the space constant of the dendrites (or spines) of the postsynaptic neuron, (iv) activation of the NMDA receptor as the cell depolarizes as a result of a reduction in voltage-dependent block of the receptor by $Mg^{2+}$ (*26*), and (v) potentiation by neuromodulators that are released during the ID (for example, norepinephrine, somatostatin, and acetylcholine) (*27*).

In addition to direct increases in excitatory synaptic efficacy, the depolarizing effects of EPSPs can be supplemented by several voltage-dependent intrinsic currents that exist in CNS neurons. These include slowly inactivating $Na^+$ and $Ca^{2+}$ currents (*28, 29*) and a large, transient $Ca^{2+}$ current that is likely to be responsible for $Ca^{2+}$-dependent action potentials (*30, 31*).

**Table 1.** Some agents that produce focal epilepsy and cellular mechanisms of action.

| Agent | Proposed cellular mechanism |
|---|---|
| Penicillin, bicuculline, picrotoxin | Block postsynaptic GABA |
| Allylglycine, 3-mercaptoproprionic acid | Block GABA synthesis |
| Ammonium salts | Block $Cl^-$ pump |
| Tetramethylammonium, 4-aminopyridine | Block $K^+$ currents |
| Barium | Enhance $Ca^{2+}$ currents<br>Block $K^+$ currents |
| Acetylcholine | Block K current<br>Block AHP<br>Block $Ca^{2+}$ currents |
| Enkephalin | Inhibit inhibitory interneurons |
| Electroconvulsive shock | ? Frequency potentiation of excitation<br>? Frequency depression of inhibition<br>Change in extracellular ionic concentrations |
| High $[K^+]_o$ | Change intrinsic membrane characteristics<br>Induce bursting<br>Depolarize neurons |
| Low $[Ca^{2+}]_o$ | Increase neuronal excitability<br>Decrease neuronal threshold |
| Cardiac glycosides | Decrease $Na^+$ pump<br>Increase $[K^+]_o$<br>Increase tendency to fire $Ca^{2+}$ spikes |
| Alumina cream | Neuronal loss<br>Neuronal distortion<br>Selective decrease in inhibitory neurons |
| Freeze, cobalt, iron salts | ? |

Thus, it can be proposed that as the potentiated EPSP begins to depolarize the neuron, a threshold is reached for the development of a slowly inactivating $Na^+$ current that amplifies the depolarization. As depolarization continues, the low threshold $Ca^{2+}$ current may turn on to further depolarize the neuron, while NMDA-mediated excitatory synapses become more effective. Eventually, both higher threshold $Na^+$ and $Ca^{2+}$ currents are activated, and the neuron discharges with a burst of action potentials and an additional slow depolarization (*32*).

This hypothesis involves the interplay of both synaptic and voltage-dependent intrinsic events that occur in normal central neurons. Epilepsy occurs when the usual balance of these normal events is altered by a change in synaptic efficacy or a change in the control of intrinsic membrane currents. The reason that any given form of epilepsy may develop in a given brain region may depend on (i) differences in densities and locations of channels on various neurons, (ii) the interaction of intrinsic currents with one another and with synaptic currents under physiological conditions, (iii) the local synaptic organization of a given area, and (iv) the liberation of endogenous synaptic modulators that may alter the various voltage-dependent membrane currents through second messenger pathways (*33*).

An alternative hypothesis for DS generation focuses more on changes in the intrinsic properties of neurons resulting in the development of burst firing independent of a primary change in synaptic interactions. In epilepsy models, this is most readily accomplished by inhibiting $K^+$ currents and by allowing the slower $Ca^{2+}$ currents to be expressed (*28*). Under more "natural" circumstances, a variety of possible mechanisms may contribute to the development of endogenous burst propensity in the absence of exogenous epileptogenic agents: (i) neuromodulators (acetylcholine, norepinephrine, and peptides) can reduce $K^+$ currents (*34*) (although stimulation of endogenous pathways, even intensely, has not been shown to produce sufficient inhibition of $K^+$ currents to result in burst firing); (ii) both elevation of extracellular K ($[K^+]_o$) and reduction of extracellular Ca ($[Ca^{2+}]_o$) can change membrane characteristics and induce burst firing modes (*35*); and (iii) anatomical distortion and redistribution of channels after injury and partial denervation as seen in chronic epileptic foci may induce burst firing.

Finally, some investigators have proposed that neurons with endogenous bursting characteristics must act as a pacemaker in order for epileptiform activity to develop (*36–40*). Such neurons would be the CA2 and CA3 pyramidal cells in the hippocampus (*38*), layer IV and superficial layer V neocortical pyramidal cells (*29, 39*), or the abnormally burst-firing neurons in chronic neocortical foci (*19, 40*). This hypothesis is supported by the demonstration of the lower threshold for the induction of interictal discharges by epileptogenic agents in CA2 and CA3 and layer IV, the spread of abnormal activity from these areas to nearby areas in some experimental foci (*29, 39*), and by the correlation of the number of bursting cells with the seizure frequency in chronic foci (*40*). However, this hypothesis has been challenged on theoretical grounds by models that demonstrate that a system with either positive or negative feedback elements does not require unstable individual elements in order to develop oscillating behavior (*41*). There is also experimental evidence against the obligatory involvement of neurons with endogenous burst-firing characteristics. Studies of in vivo hippocampal penicillin epilepsy (*42*) and in vitro low Ca–high $K^+$ models of epilepsy (*43*) indicate that area CA1 is able to develop spontaneous IDs and seizures independent of areas CA2 and CA3. In addition, neocortical and spinal cord cultures, in which individual neurons do not discharge with intrinsic bursts, become organized into small synaptic networks that show synchronized

"burst" behavior—all as a result of synaptic interactions (*44*). Thus it appears that endogenous, $Ca^{2+}$-dependent bursts are not strictly necessary for the development of synchronous bursting activity in a neural network, although their presence may be facilitatory and CNS regions containing such burst-firing neurons may have a particularly high epileptiform potential.

## "Inhibition" in and Around the Epileptic Focus

The hyperpolarization that follows the DS appears to be responsible for limiting the duration of the ID, for determining the frequency of IDs, and for preventing the deterioration of IDs into seizures. Its disappearance is often related to the onset of seizures and spread of epileptiform activity (*4, 8, 10*). Available data indicate the mechanisms responsible for the post-DS HP may differ in different cells and in different kinds of experimental foci.

Under physiological conditions, there are at least seven mechanisms that hyperpolarize neurons: (i) short-latency GABA-mediated inhibitory postsynaptic potentials (IPSPs) due to opening of $Cl^-$ channels, (ii) longer latency, slower IPSPs that may be due to opening of $K^+$ channels (*45*), (iii) several types of voltage-sensitive $K^+$ channels (*46*), (iv) $Ca^{2+}$-dependent $K^+$ channels (*47*), (v) $Ca^{2+}$-dependent $Cl^-$ channels (*48*), (vi) voltage messenger–dependent chloride current located in the hippocampal dendrites (*33*), and (vii) an electrogenic $Na^+$ pump (*49*). Various cell types have been shown to have different combinations of these conductances; thus, different mechanisms may be responsible for inhibition during epileptic events depending on the type of cells involved. For example, in nonepileptic conditions, most hippocampal neurons have a large afterhyperpolarization (AHP) after any prolonged burst discharge. This AHP is believed to be generated by a $Ca^{2+}$-dependent $K^+$ conductance (*47*). In contrast, the majority of neocortical neurons in slices and in culture do not appear to have such large $Ca^{2+}$-dependent $K^+$ conductances but rather have a more heterogeneous set of afterpotentials (*28, 29, 44*).

This kind of variability is also expressed in epileptic events (Table 2). In penicillin foci in vivo, the post-DS HP appears to be a $Cl^-$-dependent process (*5*), which suggests that it has a synaptic origin and is possibly mediated by GABA. Also, the DS in some cells may be preceded by a hyperpolarization, another suggestion that at least part of the cellular hyperpolarization is of synaptic origin (*6, 8*). Despite, or perhaps because of the availability of more refined techniques, the in vitro data have become quite confusing and a variety of $K^+$ currents have been invoked (see Table 2).

Thus, the post-DS HP in vitro is complex and is likely to be generated by several mechanisms, which at the moment defy a single unifying hypothesis. It remains to be seen whether the same complexities exist in vivo. Our view is that for most epileptic events, especially those that occur under natural circumstances, synaptic inhibition will play the critical role in limiting the size of epileptic discharges, although other currents may be involved.

## Development of Synchrony

Another crucial issue related to the development of the ID is how so many neurons within a focus develop simultaneous depolarizations. Synchronization may occur by any of several synaptic and nonsynaptic mechanisms: (i) recurrent synaptic excitation, (ii) antidromic activation of the afferent fibers, (iii) ephaptic interactions due to large currents that flow through extracellular spaces, (iv) changes in extracellular ionic concentrations, (v) electrical coupling between cortical neurons, and (vi) the diffuse liberation of modulators.

Probably the most important of these is recurrent synaptic excitation. Extensive recurrent collaterals have been demonstrated anatomically, and the excitatory nature of some of these has been demonstrated physiologically both in neocortex and in hippocampus (*50*). Activation of these connections serves as a direct and important positive-feedback mechanism, and, moreover, may provide the source for the EPSPs responsible for the DS (*8–11, 24, 50*). In addition, projected excitatory connections provide the basis for the spread of epileptic activity to normal brain. Synchronizing activity can also be provided indirectly as a result of backfiring of the axons that project to the cortical focus. These axons have large networks of collaterals, which are likely to contribute to the involvement of a large number of neurons in the discharge (*14*).

Extracellular fields created by the flow of currents between different segments of a given neuron can influence the activity of nearby neurons, and relatively large fields can develop during epileptiform activity (*51*). In addition, both the elevation of extracellular $K^+$ and the decline of extracellular $Ca^{2+}$ that occur during IDs and seizures also serve to bring neurons closer to threshold and enhance synchronizing processes. It has even been possible to provoke epileptiform discharges in hippocampal slices in the apparent absence of synaptic coupling by bathing the slices in media containing low $Ca^{2+}$ and inorganic $Ca^{2+}$ channel blockers or low $Ca^{2+}$ and high $K^+$ (*43, 52*). Presumably, such discharges occur as a result of the increased excitability of individual neurons in the altered ionic environment and ephaptic interactions, although the late involvement of synaptic activity has also been demonstrated, even under these stringent conditions (*53*). Although these extreme circumstances do not exist in normal cortex, these models serve to emphasize that several different mechanisms may play critical roles in promoting epileptiform activity.

Another potential, although still controversial, synchronizing mechanism has been postulated. Some neurons in neocortex and hippocampus are electrically coupled (*54*), but the extent of such

**Table 2.** Postulated mechanisms underlying post–depolarizing shift hyperpolarizing potential in various kinds of acute epileptic foci.

| Preparation | Epileptogenic agent | Cell type | Mechanism | References |
|---|---|---|---|---|
| Neocortex in vitro | Penicillin | | $Cl^-$-dependent, presumed synaptic | (*5*) |
| Hippocampus in vivo | Penicillin | | Synaptic event, presumed $Cl^-$-dependent | (*5, 8*) |
| Hippocampal slice | Bicuculline, picrotoxin | CA1 | $Ca^{2+}$-dependent, $K^+$ conductance | (*71*) |
| Hippocampal slice | Bicuculline, picrotoxin | CA3 | Non–$Ca^{2+}$-dependent | (*71*) |
| Hippocampal slice | Penicillin | CA1 | Several conductances | (*72, 73*) |
| Hippocampal slice | Penicillin | CA3 | Voltage-dependent, $K^+$ conductance | (*72*) |

coupling appears quite limited. Such electrical coupling may play a role in synchronization and, if so, it is possible that local changes in coupling due to physiological processes or epileptogenic agents could facilitate the development of epileptic events (*55*).

We can propose a model for the synchronizing mechanisms involved in the development of the ID. As neurons in an epileptogenic region are activated by various stimuli, the enhanced effectiveness of local recurrent excitatory connections tends to recruit nearby neurons into synchronous activity. As more neurons in a small area discharge together, the current that flows through extracellular spaces tends to further synchronize the neurons by ephaptic interactions. At the same time, both the increase in extracellular $K^+$ and the decrease in extracellular $Ca^{2+}$ that occur during the heightened neuronal activity tend to bring many neurons closer to the firing threshold and induce burst firing, both of which enhance synchronization. As the activity within the focus becomes more intense, the ionic changes become larger and more spatially dispersed, and neurons in areas around the active focus can become involved by secondary changes in their extracellular environment, even if they are not directly activated by synaptic events. The antidromic activation of axon terminals also amplifies synaptic coupling by releasing neurotransmitters from local axon collaterals. Thus, synaptic activity may initiate the process and nonsynaptic events amplify it.

This model indicates that many mechanisms can play a role in the process of synchronization, and therefore the role of the functional, anatomical, and physical arrangement of the cortical aggregate involved in the epileptic event becomes of paramount importance. Different parts of the cortex may differ in the prevalence of a specific recurrent system, the presence of a given modulator, the anatomical arrangement and orientation of the neurons within the aggregate, or the packing of the neurons within cell body layers. A corollary to this concept is that in different cortical aggregates, one or another of these synchronizing mechanisms may be most important for the production of the ID.

## Transition to Seizures and the Spread of Epileptic Activity

During the transition to seizures in acute focal models, several important events occur that allow the usually self-limited ID to spread in time and anatomic space. The most important phenomenon is the disappearance of the post-DS HP and its replacement by a depolarization (*4, 8, 10*). Surround inhibition is also reduced. Depolarizing events become prominent in surround areas and in distant areas of brain.

If the post-DS HP is a result of some form of $K^+$ current, elevations of $[K^+]_o$ that occur during the transition to seizures would change the $K^+$ equilibrium potential and reduce the hyperpolarization. Experimental data addressing this hypothesis, however, have indicated that this does not appear to be sufficient to eliminate or invert the AHP (*56*). It is also possible that a $Ca^{2+}$-dependent $K^+$ conductance could be reduced by several neuromodulators, including acetylcholine, norepinephrine, and dopamine, acting via second messenger cascades (*34*). To date, none of these agents has been shown to be powerful enough to actually eliminate the hyperpolarization under physiological conditions (that is, via physiological pathway stimulation).

On the other hand, synaptic inhibition is very labile (*57*). GABA-mediated and glycine-mediated short-latency IPSPs, and slow, presumably $K^+$-dependent IPSPs in hippocampus are all very frequency-sensitive, and decline for as yet unknown reasons with relatively low frequency activation. Postsynaptic GABA receptors also desensitize readily during excessive agonist application (*58, 59*). If the post-DS HP is generated even in part by an IPSP, it is likely to decline with repetitive activation, as do IPSPs in general. Because surround and distant inhibition are synaptic, the frequency-dependent inhibitory synaptic decrement also provides an explanation for the decline in inhibition during the spread of seizure activity.

In addition to the decrement of hyperpolarizing processes, there is a parallel increment in depolarizing events. EPSPs increase in size because of frequency potentiation. NMDA receptors may become involved in the late component of the EPSP, as the voltage-dependent block by $Mg^{2+}$ is removed (*26*). These events may be responsible for the prolongation of the DS and for the fact that EPSPs falling on the declining phase of the DS are larger than control (*10, 32*). Slow, noninactivating $Na^+$ and $Ca^{2+}$ currents may summate and contribute to the depolarization. Thus, the coincident decline of inhibition and increase of excitation, both components of normal synaptic regulation in the CNS, allow the epileptic activity to spread locally and at a distance from the original focus, in both cases directly into normal brain.

## Application of Cellular Analyses to Studies of Neural Networks

All of the hypotheses about cellular mechanisms discussed above were derived from studies of acute focal epilepsy, both in vivo and in vitro. As investigations of such mechanisms are being extended to the level of biophysics, membrane biochemistry, and molecular biology, progress is also being made in understanding seizure mechanisms at the level of local and projected neural networks. At local levels, this analysis directly incorporates current concepts about cellular mechanisms underlying IDs and seizures, whereas in other models, such as those analyzing the role of projecting networks, the cellular phenomena at the site of initiation of the epileptic activity, and the anatomic pathways of projection must be determined before investigations can proceed to the cellular level at the target structures.

For both the hippocampus and the neocortex, the local spread of epileptiform activity and the transition between interictal and ictal activity are beginning to be analyzed in the context of local circuitry and the physiology of individual cell types. In several focal models, including the disinhibited hippocampal preparation, the neurons in all hippocampal fields are capable of generating synchronized bursts (*42, 43*). In models produced by bathing the brain slice in high $K^+$, the neurons in CA2 and CA3 have a greater propensity for DS development, whereas the neurons of CA1 appear more able to develop prolonged seizure discharges in response to the synaptic input from the discharging CA2 and CA3 neurons (*60*). In addition, in solutions of low $Ca^{2+}$, hippocampal CA1 neurons develop seizurelike events in the absence of IDs (*43*). Whether differences in cellular excitability or local network interactions are responsible for the different reactions of CA2 and CA3 as compared to CA1 neurons has not yet been determined.

In the neocortex, experiments have been directed at attempting to determine whether specific lateral synaptic connections are responsible for the propagation of IDs. In bicuculline-induced foci, IDs propagate in an orderly, but nonhomogeneous fashion, and the rate of propagation can be directionally dependent. In addition, it appears that no single set of connections, or single cortical layer, is necessary as long as some continuity exists between parts of the cortex (*61*). These experiments also suggest that synaptic connections are more critical in the local propagation of epileptiform activity than are changes in extracellular $K^+$ and $Ca^{2+}$ activity, at least for this model.

Each of these studies suggests that different parts of a brain region

can participate to different degrees in the epileptic process. One area may have a low threshold for the development IDs, while another area may respond to the increased input from the first area by developing seizurelike activity, and a third area may act as a gate for the spread of the epileptiform activity. Similarly, local propagation of the epileptiform activity may occur by several different routes, and in fact the same neurons may participate in the epileptic events in different ways as the wave of abnormal excitation proceeds through the cortex (*42*). All of the preceding implies that as studies of cellular mechanisms proceed, it becomes increasingly important to define the specific cells being studied as well as their specific roles in the local cellular network and in the model of epilepsy being analyzed. This is exceedingly difficult to do in situ and has only been practical since the introduction of slice preparations. However, it must be kept in mind that the anatomic disruptions that occur when creating the slice may contribute to the patterns of epileptiform activity, and that observations made initially in slice preparations need to be tested in intact CNS before they can be fully integrated into theories of epileptogenesis.

Recently there has been a revival of studies in the generation, propagation, and control of generalized seizures, in which the role of subcortical structures has been shown to be of paramount importance. By using "pharmacological dissection" techniques, it has become possible to determine that in at least one model, pentylenetetrazol (PTZ)-induced generalized epilepsy, a few subcortical structures (the mammillary system, including mammillary bodies, mammillothalamic tract, and anterior nuclei of the thalamus) are critical for the development and propagation of the epileptic activity (*62*). Carefully placed lesions in the mammillothalamic tracts or chemicals that enhance GABA-mediated inhibition injected directly into the anterior nuclei can protect, acting as a gate, the animals from PTZ-induced seizures.

Another specific anatomic locus in the deep prepiriform cortex, called "area tempestas," appears to be involved in the development of generalized clonic seizures induced by local injection of bicuculline and potentiated by morphine (*63*). The injection of GABA agonists, NMDA-receptor antagonists, and phenytoin (*64*) in the same area protects the animal from generalized seizures induced by systemic administration of bicuculline. Thus, this area seems to have most of the characteristics of an operational focus for the generation and control of the bicuculline-induced generalized seizures.

A similar picture is developing in relation to other generalized seizures. The substantia nigra appears able to control the propagation of seizures induced by several kinds of epileptogenic stimuli, including electroshock, systemic convulsants, and kindling (*65*). Suppression of nigral output by either infusion of inhibitory agents (GABA mimetics) or by destruction, inhibits the expression of clonic seizures (*66*). A series of lesion studies and pharmacologic manipulations indicate that seizures are potentiated when the GABA-containing nigrotectal output inhibits tectal neurons, although the tectal neurons themselves are not necessary for the propagation of these seizures (*67*).

The cellular events that underlie these control mechanisms for seizure propagation remain to be determined, although the questions that need to be addressed are similar to those that have been discussed in relation to acute focal epilepsy. Do the neurons in these areas have properties that are different from those described in other structures that have been more widely studied? Are the local circuits in these regions substantially different from those of hippocampus or neocortex, so as to more readily allow the development of epileptiform activity? Is it simply the connections of these regions that make them so influential in the propagation of seizures? Studies so far have emphasized the important role of GABA as an inhibitory neurotransmitter, of the NMDA receptors for excitatory functions, and of the roles of modulatory substances such as norepinephrine and the opiate peptides, but very little is known about how these regions express their roles on the target structures, or which target structures create the generalized seizures.

The kindling model of epilepsy poses the same type of questions. It has been known for more than 15 years that specific types of nonepileptic stimuli applied to certain brain areas can cause permanent changes in excitability that lead to chronic epilepsy. A great deal is known about the phenomenon of kindling (*68*), but relatively little is known about the mechanisms that underlie the increased excitability. Currently, it is still unclear if the cellular mechanisms of kindling are similar to the cellular mechanisms underlying acute focal epilepsy (*69*).

As discussed in the beginning of this article, the use of the acute focal epilepsy model was an attempt by neuroscientists to develop a means by which at least one form of human epilepsy could be analyzed and understood at a cellular level. When even this simple animal model proved too complex for unraveling mechanisms, in vitro models were used to ask more detailed questions, and research became focused at cellular and molecular levels. At the same time, physiological and pathological studies of human epilepsy with a variety of new techniques, such as depth electroencephalography, positron emission tomography, magnetic resonance spectroscopy, receptor mapping, and neurotransmitter immunohistochemistry, have been redefining the problems of the clinical epilepsies and have been posing new questions that are challenging the relevance of many of the experimental models that were developed in the past 25 years (*18*). It is clear that more extensive and precise cellular analyses of the human disorders are needed, while at the same time new models will have to be used to answer the questions developed from the human studies. The relations between brain areas that produce IDs and those that generate seizures, the mechanisms by which seizures develop in humans, the role of GABA-mediated inhibition in both focal and generalized human epilepsy, and the effects of long-standing ictal and interictal activity on the functioning of normal areas of brain (*18, 70*) are all issues that are not well understood from analyses of simple focal epilepsy models.

## Conclusion

The picture that emerges with regard to simple models of focal epilepsy is as follows: (i) many agents with widely varying primary cellular mechanisms of action may initiate epilepsy. (ii) The individual elements of the epileptic process—the DS, the post-DS HP, the synchronicity, the spread of the epileptic event to beyond the limits of the interictal focus—all can be produced by different mechanisms depending on the experimental model studied, the properties of the neurons involved, and the anatomical and functional organization of a given cortical area. As the development of both epileptogenicity and the interictal discharge is a function of the relative importance of multiple mechanisms locally, at this time, a unified theory of epilepsy (especially one that includes complicated human epilepsy), which is based on only one or another of the possible mechanisms, is difficult to justify. In fact, just as there are different epilepsies clinically, there are liable to be different mechanisms of the epilepsies at the cellular level.

Many of the individual components of epileptic processes are becoming better characterized as we learn more about the physiological function of the brain at the cellular and molecular level. The manner by which each of the components is integrated into the epileptic process in any given model or brain structure remains a larger problem, especially as we move from simplified models to those that more closely mimic human disorders. The DS, which

forms the basis of the epileptic spike discharge, is perhaps the best understood of the cellular phenomena, especially with the realization that the relative contribution of each of its several possible components may differ in different locations.

The problems more cogent to clinical epilepsy, the manner by which the hypersynchronous activity is limited in both time and space, and the mechanisms by which such inhibitory control is overcome during the transition to seizure remain less understood. The last mentioned process is at least as important in relation to the clinical problem of epilepsy as is the initial establishment of the focal area of excessive excitability. The mechanisms and pathways by which focal epileptiform activity spreads throughout the normal CNS and the mechanisms underlying the generalized epilepsies are just beginning to be approached and require further characterization, both at the level of the local circuitry as well as that of the whole brain. The recognition of specific areas of the subcortical gray matter that act either as gates for the propagation of convulsive activity (for example, the nuclei of the anterior thalamus and the substantia nigra) or as the operational focus for generalized seizures (for example, the prepiriform area) may potentially open new avenues for therapeutic approaches. In order to understand the role of these structures, the questions to be solved use the same lexicon as for the interictal event. Finally, even with different and contemporary technical scientific concepts, there is a return to the original conceptual model of the 1950s as far as mechanisms underlying generalized seizures are concerned.

We are now in an era when our understanding of CNS cellular and synaptic function is increasing at a rapid pace. As new physiological mechanisms are discovered, new mechanisms of hyperexcitability can be postulated. It is important to recognize, however, that as new ideas about epileptogenesis evolve, older hypotheses should not be eliminated but rather should be placed into a larger conceptual framework. These hypotheses should be viewed as sequential, partial explanations of epileptic mechanisms, each in relation to the experimental model used or the specific anatomical-functional substrate of the neuronal aggregate involved in the pathological process producing the epileptic condition. No single hypothesis provides "the" common element for all epileptic conditions; each of them provides a significant contribution. Within this view, we are now in a position to use what we do understand to begin to propose new and innovative approaches to the prevention and treatment of epilepsy, even as we strive to further unravel its underlying mechanisms.

## REFERENCES AND NOTES


1. W. Penfield and H. Jasper, *Epilepsy and the Functional Anatomy of the Human Brain* (Little, Brown, Boston, 1954).
2. C. Li, *J. Neurophysiol.* **22**, 436 (1959); R. Schmidt, L. Thomas, A. Ward, Jr., *ibid.*, p. 285; A. Ward, Jr., and R. Schmidt, *Arch. Neurol.* **5**, 308 (1961); E. S. Goldensohn and D. P. Purpura, *Science* **139**, 840 (1963); M. Sawa, N. Maruyama, S. Kaji, *Electroencephalogr. Clin. Neurophysiol.* **15**, 209 (1963); M. Sawa, S. Kaji, K. Usuki, *ibid.* **19**, 248 (1965); M. Sawa, N. Maruyama, S. Kaji, K. Nakamura, *Jpn. J. Physiol.* **16**, 126 (1966).
3. H. Matsumoto and C. Ajmone-Marsan, *Exp. Neurol.* **9**, 286 (1964).
4. ———, *ibid.*, p. 305.
5. D. Prince, *ibid.* **21**, 467 (1967); *ibid.* **22**, 307 (1968).
6. ———, *Annu. Rev. Neurosci.* **1**, 395 (1978); *Epilepsia* **26** (suppl. 1), S3 (1985).
7. M. Dichter and W. A. Spencer, *J. Neurophysiol.* **32**, 649 (1969).
8. ———, *ibid.*, p. 663.
9. H. Matsumoto, G. F. Ayala, R. Gumnit, *ibid.*, p. 688.
10. G. F. Ayala, H. Matsumoto, R. Gumnit, *ibid.* **33**, 73 (1970).
11. G. F. Ayala, M. Dichter, R. Gumnit, H. Matsumoto, W. A. Spencer, *Brain Res.* **52**, 1 (1973).
12. D. Prince and B. Wilder, *Arch. Neurol.* **16**, 194 (1967).
13. P. Schwartzkroin *et al.*, *Brain Res.* **210**, 59 (1975); P. Schwartzkroin, R. Mutani, D. Prince, *J. Neurophysiol.* **39**, 795 (1975).
14. M. J. Gutnick and D. A. Prince, *Science* **176**, 424 (1972); J. Noebels and D. Prince, *J. Neurophysiol.* **41**, 1267 (1978).
15. M. Dichter, C. Herman, M. Selzer, *Brain Res.* **48**, 173 (1972).
16. A. Fertziger and J. Ranck, *Exp. Neurol.* **26**, 571 (1970); W. Moody, K. Futamachi, D. Prince, *ibid.* **42**, 248 (1974); B. Ransom, *Brain Res.* **69**, 83 (1974).
17. U. Heinemann, H. Lux, M. Gutnick, *Exp. Brain Res.* **27**, 237 (1977).
18. H. Wieser, *Electroclinical Features of the Psychomotor Seizure* (Butterworths, London, 1983); J. Engel, in *Mechanisms of Epilepsy: The Transition to Seizure*, M. Dichter, Ed. (Plenum, New York, in press).
19. A. Ward, Jr., *Adv. Neurol.* **34**, 189 (1983).
20. C. E. Ribak, A. B. Harris, J. E. Vaughn, E. Roberts, *Science* **205**, 211 (1979); C. E. Ribak, R. M. Bradburne, A. B. Harris, *J. Neurosci.* **2**, 1725 (1982).
21. D. Prince and K. Futamachi, *Electroencephalogr. Clin. Neurophysiol.* **29**, 496 (1970).
22. "Normal" neurons are those that have not been directly exposed to the epileptogenic agent or pathological process. This would include neurons that have experienced unusual degrees of chronic activation as a result of synaptic input from "spike" foci, even though, after time, the physiology of such neurons may be changed.
23. G. F. Ayala and C. Vasconetto, *Electroencephalogr. Clin. Neurophysiol.* **33**, 96 (1972).
24. D. Johnston and T. H. Brown, *Science* **211**, 294 (1981).
25. P. Andersen and T. Lomo, in *Structure and Function of Inhibitory Neuronal Mechanisms*, C. Von Euler, S. Skoglund, U. Soderberg, Eds. (Pergamon, Oxford, U.K., 1968), pp. 335–342; C. Yamamoto, K. Matsumoto, M. Takagi, *Exp. Brain Res.* **38**, 469 (1980); S. Erulkar, *Rev. Physiol. Biochem. Pharmacol.* **98**, 63 (1985); C. E. Herron, R. A. J. Lester, E. J. Coan, G. L. Collingridge, *Nature (London)* **322**, 265 (1986).
26. M. Mayer and G. Westbrook, *J. Physiol. (London)* **361**, 65 (1985).
27. M. Dichter and J. Delfs, in *Neurosecretion and Brain Peptides*, J. Martin, S. Reichlin, K. Bick, Eds. (Raven, New York, 1981); pp. 145–157; P. Stanfield, Y. Nakajima, K. Yamaguchi, *Nature (London)* **315**, 498 (1985).
28. In neocortex: C. Stafstrom, P. Schwindt, W. Crill, *Brain Res.* **236**, 221 (1982); C. Stafstrom, P. Schwindt, J. Flatman, W. Crill, *J. Neurophysiol.* **52**, 244 (1984); C. Stafstrom, P. Schwindt, M. Chubb, W. Crill, *ibid.* **53**, 153 (1985); M. Dichter *et al.*, *Soc. Neurosci. Abstr.* **12**, 1344 (1986); C. Zona *et al.*, *ibid.*, p. 1345. In hippocampus: D. Johnston, J. J. Hablitz, W. A. Wilson, *Nature (London)* **286**, 391 (1980); D. Brown and W. Griffiths, *J. Physiol. (London)* **337**, 303 (1983).
29. B. Connors, M. Gutnick, D. Prince, *J. Neurophysiol.* **48**, 1302 (1982).
30. In neocortex: M. Dichter, J. Lisak, B. Biales, *Brain Res.* **289**, 99 (1983). In hippocampus: P. Schwartzkroin and M. Slawsky, *ibid.* **135**, 157 (1977); R. K. S. Wong, D. A. Prince, A. I. Basbaum, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 986 (1979).
31. R. Wong and D. Prince, *Brain Res.* **159**, 385 (1978).
32. C. Herron, R. Williamson, G. Collingridge, *Neurosci. Lett.* **61**, 255 (1985); R. Dingledine, M. Hynes, G. King, *J. Physiol. (London)* **380**, 175 (1986).
33. H. Higashida and D. A. Brown, *Nature (London)* **323**, 333 (1986); D. V. Madison, R. C. Malenka, R. A. Nicoll, *ibid.* **321**, 695 (1986).
34. Acetylcholine: D. Brown, P. Adams, A. Constanti, *J. Auton. Nerv. Syst.* **6**, 23 (1982); L. Benardo and D. Prince, *Brain Res.* **249**, 333 (1982); A. Cole and R. Nicoll, *Science* **221**, 1299 (1983); *J. Physiol. (London)* **352**, 173 (1984); D. Madison, B. Lancaster, R. Nicoll, P. Adams, *Soc. Neurosci. Abstr.* **11**, 567 (1985). Norepinephrine: D. V. Madison and R. A. Nicoll, *Nature (London)* **299**, 636 (1982). Dopamine: R. Malenka and R. Nicoll, *Soc. Neurosci. Abstr.* **11**, 467 (1985), but see: L. S. Benardo and D. A. Prince, *Nature (London)* **297**, 76 (1982); *J. Neurosci.* **2**, 415 (1982) for differing view of dopamine's effects.
35. W. Crill, P. Schwindt, G. Tribble, in *Physiology and Pharmacology of Epileptogenic Phenomena*, M. Klee *et al.*, Eds. (Raven, New York, 1982), pp. 189–195.
36. P. Schwartzkroin and A. Wyler, *Ann. Neurol.* **7**, 95 (1980).
37. M. Gutnick, B. Connors, D. Prince, *J. Neurophysiol.* **48**, 1321 (1982); R. Traub and R. Wong, *ibid.* **49**, 459 (1983); B. Alger, in *Brain Slices*, R. Dingledine, Ed. (Plenum, New York, 1984), pp. 155–199; W. Knowles, R. Traub, R. Wong, R. Miles, *Trends Neurosci.* **8**, 73 (1985).
38. P. Schwartzkroin and D. Prince, *Brain Res.* **147**, 117 (1978); R. Wong and R. Traub, *J. Neurophysiol.* **49**, 442 (1983); R. Miles, R. Wong, R. Traub, *Neuroscience* **12**, 1179 (1984).
39. B. W. Connors, *Nature (London)* **310**, 685 (1984).
40. A. Wyler, K. Burchiel, A. Ward, Jr., *Epilepsia* **19**, 475 (1978).
41. D. Johnston and T. Brown, *Adv. Neurol.* **44**, 263 (1986).
42. M. Dichter, C. Herman, M. Selzer, *Electroencephalogr. Clin. Neurophysiol.* **34**, 619 (1973); *ibid.*, p. 631.
43. A. Konnerth, U. Heinemann, Y. Yaari, *Nature (London)* **307**, 69 (1984); *J. Neurophysiol.* **56**, 409 (1986); Y. Yaari *et al.*, *ibid.*, p. 424.
44. M. Dichter, *Brain Res.* **149**, 279 (1978); R. Macdonald and J. Barker, *Neurology* **28**, 325 (1978).
45. R. Thalmann, E. Peck, G. F. Ayala, *Neurosci. Lett.* **21**, 319 (1981); R. A. Nicoll and B. E. Alger, *Science* **212**, 957 (1981); B. Alger, *J. Neurophysiol.* **52**, 892 (1984).
46. B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, 1984).
47. R. Meech, *Annu. Rev. Biophys. Bioeng.* **7**, 1 (1978); J. Hotson and D. Prince, *J. Neurophysiol.* **43**, 409 (1980).
48. M. Barish, *J. Physiol. (London)* **342**, 309 (1983); D. Owen, M. Segal, J. L. Barker, *Nature (London)* **311**, 567 (1984); M. Mayer, *J. Physiol. (London)* **364**, 217 (1985).
49. R. Thomas, *Physiol. Rev.* **52**, 563 (1972).
50. K. Takahashi, K. Kubota, M. Uno, *J. Neurophysiol.* **30**, 22 (1967); R. Lebovitz, M. Dichter, W. A. Spencer, *Int. J. Neurosci.* **2**, 99 (1971); B. MacVicar and F. Dudek, *Brain Res.* **184**, 220 (1980); W. Knowles and P. Schwartzkroin, *J. Neurosci.* **1**, 318 (1981); R. Miles and R. Wong, *J. Physiol. (London)* **373**, 397 (1986).
51. V. Markin, *Biofizika* **15**, 120 (1970); K. Maeda, T. Yagi, A. Noguchi, *IEEE Trans. Biomed. Eng.* **27**, 139 (1980); R. C. Malenka, J. D. Kocsis, B. R. Ransom, S. G. Waxman, *Science* **214**, 339 (1981); C. Taylor and F. Dudek, *J. Neurophysiol.* **52**, 126 (1984); C. Yim, K. Krnjevic, T. Dalkara, *ibid.* **56**, 99 (1986).
52. J. G. R. Jefferys and H. L. Haas, *Nature (London)* **300**, 448 (1982); C. P. Taylor and F. E. Dudek, *Science* **218**, 810 (1982); Y. Yaari, A. Konnerth, U. Heinemann, *Exp. Brain Res.* **51**, 153 (1983); C. Taylor and F. Dudek, *J. Neurophysiol.* **52**, 143 (1984).
53. A. Konnerth and U. Heinemann, *Neurosci. Lett.* **42**, 255 (1983).
54. B. MacVicar and F. Dudek, *Brain Res.* **196**, 494 (1980); M. J. Gutnick and D. A. Prince, *Science* **211**, 67 (1981); B. MacVicar, N. Ropert, K. Krnjevic, *Brain Res.* **238**, 239 (1982); H. Schmalbruch and H. Jahnsen, *ibid.* **217**, 175 (1981).
55. S. G. Rayport and E. R. Kandel, *Science* **213**, 462 (1981); D. Spray, *Annu. Rev. Physiol.* **47**, 281 (1985).
56. V. Tancredi, C. Franck, M. Dichter, *Soc. Neurosci. Abstr.* **12**, 1345 (1986).

57. GABA-mediated IPSPs: Y. Ben-Ari, K. Krnjevic, W. Reinhardt, *Can. J. Physiol. Pharmacol.* **57**, 1462 (1979); R. Wong and D. Watkins, *J. Neurophysiol.* **48**, 938 (1982); M. McCarren and B. Alger, *ibid.* **53**, 557 (1985). Glycine-mediated IPSPs: H. Korn *et al.*, *J. Neurosci.* **4**, 125 (1984).
58. K. Krnjevic, in *Physiology and Pharmacology of Epileptogenic Phenomena*, M. Klee, D. Lux, E. Speckman, Eds. (Raven, New York, 1982), pp. 123–130; D. Barnes and M. Dichter, *Soc. Neurosci. Abstr.* **10**, 419 (1984).
59. M. Frosch, thesis, Harvard University (1987).
60. R. Traynelis and R. Dingledine, *Soc. Neurosci. Abstr.* **12**, 74 (1986).
61. B. Connors and R. Chervin, *ibid.*, p. 350; R. Chervin and B. Connors, *ibid.*, p. 350.
62. M. A. Mirski and J. A. Ferrendelli, *Science* **226**, 72 (1984); *Epilepsia* **25**, 661 (1984); J. Miller and J. Ferrendelli, in *Mechanisms of Epilepsy: The Transition to Seizure*, M. Dichter, Ed. (Plenum, New York, in press).
63. S. Piredda, C. Lim, K. Gale, *Life Sci.* **36**, 1295 (1985).
64. S. Piredda and K. Gale, *Nature (London)* **317**, 623 (1985).
65. M. J. Iadarola and K. Gale, *Mol. Cell. Biochem.* **39**, 305 (1981); *Science* **218**, 1237 (1982); G. Le Gal La Salle, M. Kijima, S. Feldblum, *Neurosci. Lett.* **36**, 69 (1983); J. McNamara, L. Rigsbee, M. Galloway, *Eur. J. Pharmacol.* **86**, 485 (1983); J. McNamara, M. Galloway, L. Rigsbee, C. Shin, *J. Neurosci.* **4**, 2410 (1984).
66. D. Garant and K. Gale, *Brain Res.* **273**, 156 (1983); K. Gale, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 2414 (1985).
67. D. Garant and K. Gale, *Soc. Neurosci. Abstr.* **12**, 80 (1986).
68. G. Goddard, *Nature (London)* **214**, 1020 (1967); R. Racine, *Neurosurgery* **3**, 234 (1978); J. McNamara *et al.*, *Crit. Rev. Clin. Neurobiol.* **1**, 341 (1985).
69. G. King *et al.*, *J. Neurophysiol.* **54**, 1295 (1985); W. Wadman *et al.*, *Exp. Brain Res.* **57**, 404 (1985); D. McIntyre and R. Wong, *Exp. Neurol.* **88**, 529 (1985); *J. Neurophysiol.* **55**, 1295 (1986); S. Piredda *et al.*, *Exp. Neurol.* **90**, 659 (1986); J. McNamara, D. Bonhaus, C. Shin, in *Mechanisms of Epilepsy: The Transition to Seizure*, M. Dichter, Ed. (Plenum, New York, in press).
70. J. Engel and C. Wilson, in *Neurotransmitters, Seizures, and Epilepsy III*, P. L. Morselli, G. Nistico, K. G. Lloyd, R. G. Fariello, J. Engel, Jr., Eds. (Raven, New York, 1986), pp. 1–13.
71. B. E. Alger and R. A Nicoll, *Science* **210**, 1122 (1980).
72. P. A. Schwartzkroin and C. E. Stafstrom, *ibid.*, p. 1125.
73. J. Hablitz, *Neurosci. Lett.* **22**, 159 (1981).

## Research Articles

# Laser-Stimulated Luminescence Used to Measure X-ray Diffraction of a Contracting Striated Muscle

YOSHIYUKI AMEMIYA, KATSUZO WAKABAYASHI, HIDEHIRO TANAKA, YUTAKA UENO, JUNJI MIYAHARA

**An integrating x-ray area detector that operates on the basis of laser-stimulated luminescence was used in a diffraction study of muscle contraction. The area detector has a dynamic range of 1 to $10^5$, a sensitivity about 60 times greater with approximately 1/300 as much fog background as x-ray film. It is erasable and reusable but, like film, can integrate at a practically unlimited counting rate. The high sensitivity and wide dynamic range of the detector resulted in a sufficient reduction in the exposure time to make possible the recording of a clear x-ray diffraction pattern, with up to 2.0-nanometer axial spacing, from a contracting frog skeletal muscle in as little as 10 seconds with synchrotron radiation. During the isometric contraction of the muscle, most of the actin diffraction lines increased in intensity without noticeable changes in their peak positions. Changes also occurred in diffraction intensities from the myosin heads. The results indicate that during contraction the structure of the actin filaments differs from that in the rigor state, suggesting a possible structural change in the actin subunits themselves; the myosin heads during contraction retain the axial periodicity of the myosin filament and become aligned in a more perpendicular manner to the actin filaments.**

X-RAY DIFFRACTION PATTERNS FROM CONTRACTING MUSCLES have been previously studied with synchrotron radiation in the time-resolved mode with gas-type one-dimensional detectors (*1–6*). These studies have provided information on the molecular changes that occur during force development and sliding movement. The approach complementary to the time-resolved structure analysis has been to study the entire two-dimensional diffraction pattern at high spatial resolution with area detectors. Such experiments had been performed by recording on film the patterns from a long series of contractions (*7–9*). However, the exposure time required for satisfactory patterns was too long, and physiological fatigue in the contracting muscle could not be avoided. X-ray television (*1, 10*) and pulse-type area detectors, such as multiwire proportional chambers (MWPC) (*11, 12*), are now being used for this purpose; thus, the required exposure time has become much shorter. These methods still do not overcome several problems. The dynamic range of x-ray television is insufficient, and the spatial distortion and nonuniformity of response cannot be neglected. The spatial resolution of MWPC is not satisfactory and the counting-rate limitation prohibits an effective use of a high x-ray flux of synchrotron radiation.

A storage phosphor screen, called "an imaging plate," and an imaging system in which it is used were recently developed for diagnostic radiography (*13*). This imaging plate is a new type of integrating area detector that can record the diffraction of synchrotron radiation during muscle contraction. We were able to measure intensity changes of weak reflections that have the periodicity of the actin filament.

**New integrating area detector**. The detector system comprises (i) a phosphor screen (the imaging plate), (ii) an image reader, (iii) an image processor, and (iv) an image writer (Fig. 1). The imaging plate (IP) is a flexible plastic plate coated with a 0.15-mm-thick layer

Y. Amemiya is at the Photon Factory, National Laboratory for High Energy Physics, Oho-machi, Tsukuba-gun, Ibaraki 305, Japan. K. Wakabayashi and Y. Ueno are at the Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan. H. Tanaka is at the Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan. J. Miyahara is a senior research staff of the Miyanodai Development Center, Fuji Photo Film Company Limited, Kaisei-machi, Ashigarakami-gun, Kanagawa 258, Japan.