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# Preface

Epilepsy is an episodic neurological disorder that has afflicted humankind throughout recorded history; yet, throughout the millennia, it has never been properly acknowledged as a disease with a biological basis. In ancient times, epilepsy was referred to, somewhat ironically, as the “Sacred Disease,” as it was imbued with negative references to the supernatural. Epilepsy was later believed to represent a form of demonic possession and thus resulted in social stigmatization and persecution. It was only late in the 19th century that epilepsy began its long and arduous journey to being justly recognized as a physical illness with complex pathophysiological substrates. Even today, the public is not fully apprised of the true nature of the epilepsies (as they are now considered), and efforts to expand awareness of this condition have been thwarted in large measure by deeply rooted preconceptions promulgated through the ages.

Within the last half-century, significant progress has been made in our basic understanding of the epileptic brain. Pivotal advances in drug development and surgical techniques, as well as the emergence of innovative approaches such as electrical stimulation of the nervous system, have led to a substantial reduction in the morbidity and mortality of patients with epilepsy (both children and adults). At the same time, remarkable developments in the basic neurosciences have enhanced our understanding of brain structure and function at ever finer levels of molecular, cellular, and genetic detail.

The intrinsic complexities associated with attempts at understanding normal brain structure and function lie at the heart of the challenges investigators face in deciphering the epileptic brain. The development of universally effective therapeutic approaches for epilepsy patients has been the elusive goal of clinicians and researchers since the early twentieth century. Yet, despite the availability of many new pharmacological agents within the last generation, at least one third of the people with epilepsy remain refractory to medical therapy, and an even smaller number of these individuals are potential candidates for epilepsy surgery. It is this last frustrating reality that has been the focus of many professionals in the epilepsy field.

Within the research arena, increasing focus has been placed on “translational” research (i.e., that which bridges the gap between the laboratory and patient bedside); however, effective communication and interchange between clinicians and basic researchers have been difficult to achieve on a widespread basis. It is clear that such interaction is paramount in the development of novel treatments based on a detailed knowledge of fundamental mechanisms. This volume incorporates new translational advances in bringing epilepsy therapies from the laboratory bench to the bedside and back again.

We wish to collectively thank our mentors, colleagues, students, and, most of all, our patients and their families for providing the inspiration and encouragement to help facilitate this “translational” dialog. Additionally, we thank the publisher and our families for the support they have given us throughout this project. Finally, we acknowledge the expert editorial and administrative assistance provided by Pat Roberson and Heather Milligan.

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# Introduction

Epilepsy is a common episodic neurological condition that is heterogeneous in its clinical presentation, yet characterized at a more fundamental level by the common denominators of neuronal hyperexcitability and hypersynchrony. Our understanding of epilepsy has advanced significantly over the past several decades, and the treatment options (both medical and surgical) have expanded greatly as well. Progress in the basic neurosciences has translated to ever-growing observations of molecular and cellular changes that are associated with the epileptic condition, some of which may be critical to the processes of epileptogenesis, such as the pathological changes that ensue over a latent period, ultimately resulting in spontaneous recurrent seizures and their negative consequences. Further, within the past decade, advances in molecular genetics have defined not only more clearly the role of seizure susceptibility genes and multigene influences, but also genetic mutations that are specifically linked to certain, albeit rare, epilepsy syndromes.

Nevertheless, despite such exciting developments, the clinical practice of epilepsy remains largely empiric, and few insights from the research bench have had meaningful clinical impact. The relative dearth of true “translational” (i.e., clinic to bench and back to clinic) research has hampered our ability to move beyond the limited trial-and-error approach of antiepileptic drug (AED) therapy to one that is based on a detailed understanding of how specific molecular changes might dictate truly rational and targeted pharmacotherapy. Yet, despite this, and even as the mainstay of epilepsy therapy continues to be represented by AEDs, clinicians have brought forth other novel drug approaches and nonpharmacological considerations to the treatment armamentarium, including innovative surgical interventions (e.g., deep brain stimulation).

Many books deal with the subject of epilepsy, with some focusing on clinical diagnosis and treatments and others exploring the pathological substrates of the various epilepsies. Also, some noteworthy volumes provide comprehensive overviews and discussions about both basic science and clinical topics in the field of epilepsy. However, there remains a need for additional references that integrate the most relevant research developments with clinical issues that impact directly on therapeutics. Such books would define the scientific basis of clinical practice and pose a set of challenging questions and considerations that could help shape not only the future of clinical research but also provide novel insights and avenues into more fundamental investigations that would yet again make us go “back to the bench and return to the clinic.” Thoughtful clinicians, who can appreciate insights drawn from the fundamental neurosciences, can and should take a more rational approach toward the treatment of patients with epilepsy. Incorporating exciting research developments into their knowledge base will empower clinicians to “think outside the box” and to test clinical hypotheses derived from implications of basic research findings.

This volume is divided into six sections. The first section begins with a broad overview of the basic anatomic and functional substrates of seizure genesis. This is followed by half a dozen chapters highlighting novel pathogenic concepts that have both emerged and have been validated experimentally. These include (1) the role of the blood–brain barrier, (2) central nervous system inflammation, (3) the critical role of metabolism in seizure genesis, (4) the mechanistic basis of drug resistance in epilepsy, (5) complex genetics underlying epileptic conditions, and (6) the unique pathophysiological basis of certain developmental epilepsies.

Chapters in the second section are related to antiepileptic drug therapy and include a current discussion on the molecular targets of AED action and the possibility that certain AEDs may exert protective effects on the disease process itself (rather than simply suppress recurrent seizures). Other considerations in the use of AEDs, both clinically available and investigational, include an appreciation for nonsynaptic mechanisms yielding potent anticonvulsant effects, pharmacokinetic

and pharmacodynamic effects, and the genetic underpinnings of AED treatment and development. Finally, with a better understanding of drug interactions and attendant toxicities, beyond what can be established as mechanisms explaining clinical efficacy, the clinician can undoubtedly optimize the long-term care of patients suffering from epilepsy.

The third section focuses on surgical treatments for epilepsy (resective or otherwise), beginning with advances in the fields of structural and functional neuroimaging which have helped enormously in the selection of epilepsy surgery candidates and improving postsurgical outcomes. At the same time, there are emerging approaches for nonsurgical ablation of epileptic tissue and a greater understanding of the molecular and cellular bases of seizure genesis based on studies of such tissues. The fourth section reviews the variety of nontraditional therapeutic options, many of which have established efficacy in the treatment of medically refractory epilepsies (such as the ketogenic diet and the vagus nerve stimulator), but the particular clinical niches of others remain to be defined (e.g., immunomodulators, neurosteroids, herbs, botanicals).

The fifth section deals with neuroendocrine, hormonal, and biobehavioral factors that influence seizure susceptibility—information that should be incorporated into the design of treatment algorithms on an individualized basis. Finally, the last section of this book provides a glimpse of what future epilepsy therapies might look like, from novel mechanisms of drug delivery to gene and stem-cell therapies for epilepsy to seizure detection methods, which provide the pretext for highly targeted and early preventative intervention. Along these lines, the final chapter provides an overview of what has become the holy grail of epilepsy therapeutics over the past decade: the goal of preventing epilepsy itself by first identifying populations at risk and, perhaps more importantly, the critical mediators and influences that in a causal manner produce an enduring epileptic condition. Such knowledge would then be employed to intervene during critical windows of disease ontogeny, as well as during brain development.

The idea for this book was inspired by our collective desire to promote bridging of the so-called “translational divide”—that is, covering innovative treatment strategies based on scientific principles that have yet to be tested rigorously in the clinical setting but yet may provide practitioners with new approaches toward epilepsy therapeutics. It is in this spirit that we earnestly hope that the reader will benefit from this volume.

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# 1 Pathophysiological Mechanisms of Seizures and Epilepsy: A Primer

Carl E. Stafstrom

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## INTRODUCTION

This chapter reviews the cellular and synaptic basis for focal and generalized seizure generation with an emphasis on ion channels and synaptic physiology. This background is useful for understanding the scientific basis of epilepsy and its treatment, as discussed in greater detail in subsequent chapters of this book.

A *seizure*, or *epileptic seizure*, is a temporary disruption of brain function due to the excessive, abnormal discharge of cortical neurons. The clinical manifestations of a seizure depend on the specific region and extent of brain involvement and may include an alteration in alertness, motor function, sensory perception, or autonomic function, or all of these. Any person can experience a seizure in the appropriate clinical setting (e.g., meningitis, hypoglycemia), attesting to the innate capacity of even a normal brain to support epileptic discharges, at least temporarily. *Epilepsy* is the condition of recurrent (two or more), unprovoked seizures, usually due to a genetic predisposition or chronic acquired pathologic state (e.g., cerebral dysgenesis, brain trauma). *Epilepsy syndrome*

refers to a constellation of clinical characteristics that consistently occur together, with seizures as a primary manifestation. Features of an epilepsy syndrome might include similar age of onset, electroencephalogram (EEG) findings, etiology, inheritance pattern, natural history of the symptoms, and response to particular antiepileptic drugs. Mechanisms leading to the generation of a seizure (*ictogenesis*) may differ from those predisposing to epilepsy, the condition of recurrent, unprovoked seizures (i.e., *epileptogenesis*) (Dichter, 2009).

A seizure is characterized by aberrant electrical activity within the brain. Such electrical activity is the net product of biochemical processes at the cellular and subcellular levels occurring in the context of large neuronal networks. Seizures often involve interplay between cortical and subcortical structures (Blumenfeld, 2003). The surface EEG is the primary clinical tool with which normal and abnormal electrical activity in the brain is measured.

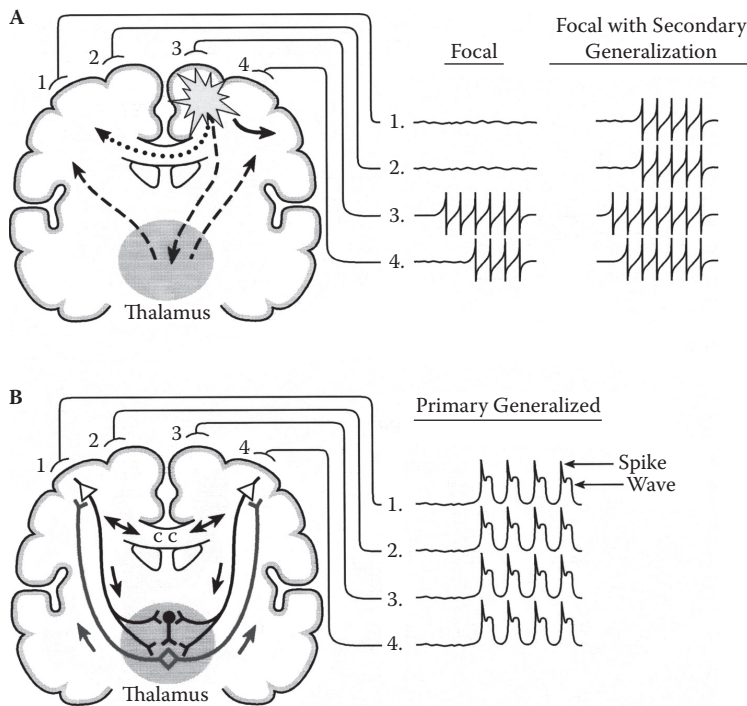
At the cellular level, the two hallmark features of epileptic activity are neuronal hyperexcitability and neuronal hypersynchrony. *Hyperexcitability* is the abnormal responsiveness (e.g., lower threshold) of a neuron to excitatory input; a hyperexcitable neuron tends to fire bursts of multiple action potentials instead of just one or two. *Hypersynchrony* refers to the recruitment of large numbers of neighboring neurons into an abnormal firing mode. Ultimately, a seizure is a network phenomenon that requires participation of many neurons firing synchronously. Conventional EEG techniques can detect cortical areas exhibiting hypersynchronous discharges in the form of interictal sharp waves or spikes. Using specialized EEG recording techniques in humans and animals with epilepsy, bursts of very localized discharges have been detected that are not detected by usual EEG methods (Engel et al., 2009). These so-called “fast ripples” (250 to 600 Hz) reflect abnormal interictal discharges in restricted cortical areas which could synchronize and lead to a seizure (see Chapter 21, this volume).

## CLASSIFICATION OF SEIZURE TYPES AND EPILEPSY SYNDROMES

Epileptic seizures are broadly divided into two groups, depending on their site of origin and pattern of spread (Figure 1.1). Partial seizures arise from a localized region of the brain, and the associated clinical manifestations relate to the function ordinarily subserved by that area. Focal discharges can spread locally through synaptic and nonsynaptic mechanisms or propagate distally to subcortical structures or through commissural pathways to involve the entire cortex. A seizure arising from the left motor cortex, for example, may cause jerking movements of the right upper extremity. If epileptic discharges subsequently spread to adjacent areas and eventually encompass the entire brain, a secondarily generalized seizure may ensue.

In contrast, generalized seizures begin with abnormal electrical discharges in both hemispheres simultaneously. Thus, the EEG signature of a primary generalized seizure is bilateral synchronous spike-wave discharges seen across all scalp electrodes. Primary generalized seizures critically involve reciprocal thalamocortical connections. The manifestations of such generalized epileptic activity can range from brief impairment of consciousness (as in an absence seizure) to rhythmic jerking movements of all extremities accompanied by loss of posture and consciousness (as in a generalized tonic-clonic seizure).

Although different mechanisms underlie partial vs. generalized seizures, it is useful to view any seizure activity as a perturbation in the normal balance between neuronal inhibition and neuronal excitation. Such an excitation/inhibition imbalance may occur in a localized region of brain, in multiple brain areas (which might be linked into a multinodal network), or simultaneously throughout the whole brain (McCormick and Contreras, 2001; Faingold, 2004). This imbalance is likely the consequence of a combination of increased excitation and decreased inhibition. It is useful to conceptualize excitation/inhibition imbalance as critical for seizures and epilepsy, but this notion may be overly simplistic when brain microcircuitry is analyzed in detail. In some circumstances, for example, increased inhibition can lead to enhanced hyperexcitability (see Inhibitory Synaptic Transmission section, later in this chapter) (Mann and Mody, 2008; Yu et al., 2006).



**FIGURE 1.1** Coronal brain sections depicting seizure types and potential routes of seizure spread. (A) Focal area of hyperexcitability (star under electrode 3) and spread to adjacent neocortex (solid arrow under electrode 4), via corpus callosum (dotted arrow) or other commissural pathways to the contralateral cerebral hemisphere or via subcortical pathways (e.g., thalamus, upward dashed arrows). Accompanying EEG patterns show brain electrical activity under electrodes 1 to 4. Focal epileptiform activity is maximal at electrode 3 and is also seen at electrode 4 (left traces). If a seizure secondarily generalizes, activity may be seen synchronously at all electrodes, after a delay (right traces). (B) Primary generalized seizure begins simultaneously in both hemispheres. The characteristic bilateral synchronous spike-wave pattern on EEG is generated by reciprocal interactions between the cortex and thalamus, with rapid spread via corpus callosum (CC) contributing to the rapid bilateral synchrony. One type of thalamic neuron (dark neuron) is a GABAergic inhibitory cell that displays intrinsic pacemaker activity. Cortical neurons (open triangles) send impulses to both thalamic relay neurons (open diamond) and to inhibitory neurons, setting up oscillations of excitatory and inhibitory activity and giving rise to the rhythmic spike waves on EEG. (From Stafstrom, C.E., in *Epilepsy and the Ketogenic Diet*, Stafstrom, C.E. and Rho, J.M., Eds., Humana Press, Totowa, NJ, 2004, p. 3. With permission.)

It is important to recognize that epilepsy is not a singular disease but rather a heterogeneous spectrum in terms of clinical expression, underlying etiologies, and pathophysiology. As such, specific mechanisms and pathways underlying specific seizure phenotypes may vary when perturbations at a given hierarchical level lead to structural and functional changes at either higher (e.g., network) or lower (e.g., molecular) levels of analysis.

## CELLULAR ELECTROPHYSIOLOGY

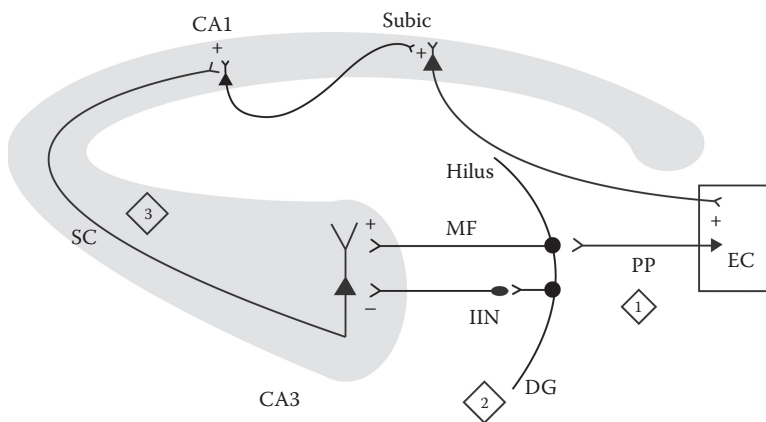
### REGIONAL DIFFERENCES IN EXCITABILITY

Brain regions differ in their intrinsic propensity to generate and propagate seizure activity, based on factors such as cell density, intrinsic membrane properties, laminar arrangement of neurons, and pattern of cellular interconnectivity. Even within the same brain region, physiological differences among various neuron types endow the region with variable excitability (Steriade, 2004).



The neocortex and hippocampus are especially prone to generating seizures. The hippocampal formation has been investigated extensively with regard to basic and epilepsy-related electrophysiological studies, and hippocampal pyramidal cells are among the most intensively studied cell types in the central nervous system. The orderly and relatively simple organization of hippocampal circuits makes them amenable for studying synaptic and nonsynaptic mechanisms relevant to seizure genesis. Furthermore, the intrinsic ability of neurons in the CA3 to fire action potentials in bursts augments the hyperexcitability of this circuit (Traub et al., 1991). Details regarding the electrophysiology of the hippocampal formation are found in classic reviews (Schwartzkroin and Mueller, 1987).

The hippocampal formation consists of the dentate gyrus, the hippocampus proper (Ammon's horn, with subregions CA1, CA2, and CA3), the subiculum, and the entorhinal cortex (Figure 1.2). These four regions are linked by excitatory, unidirectional feedforward connections. There are also some reverse projections from the entorhinal cortex to Ammon's horn and from CA3 to the dentate gyrus. The predominant forward-projecting trisynaptic circuit begins with neurons in layer II of the entorhinal cortex which project axons to the dentate gyrus along the perforant pathway, where they synapse on granule cell (and interneuron) dendrites. Granule cells, the principal cell type of the dentate gyrus, send their axons, called *mossy fibers*, to synapse on cells in the hilus and in the CA3 field of Ammon's horn. Several classes of inhibitory interneurons within the dentate hilus modulate ongoing excitatory neural activity (Lawrence and McBain, 2003). CA3 pyramidal cells project to other CA3 pyramidal cells via local collaterals, to the CA1 field of Ammon's horn via Schaffer collaterals, and to the contralateral hippocampus. CA1 pyramidal cells send their axons into the subiculum, and neurons of the subiculum project to the entorhinal cortex (as well as to other cortical and subcortical targets), thus completing the circuit. For this reason, limbic system structures such as hippocampus, subiculum, and entorhinal cortex are endowed with structural and functional features that predispose them to seizures and epilepsy (Jutila et al., 2002; Sloviter, 2008; Stafstrom, 2005).



**FIGURE 1.2** Schematic of hippocampal circuitry. Major pathways of excitatory transmission in the hippocampal trisynaptic pathway begin in neurons of entorhinal cortex (EC). EC neurons send axons to the dentate gyrus (DG) via the perforant path (PP) (1), where they synapse on granule cell dendrites. Dentate granule cells project their axons (mossy fibers [MF]) (2) to synapse on cells of the hilus (particularly inhibitory interneurons [IIN]) and in the CA3 field of Ammon's horn. CA3 pyramidal neurons then project to neurons of the CA1 field of Ammon's horn via Schaffer collaterals (SC) (3). Finally, CA1 neurons send projections outward through the fornix to other brain regions, including the subiculum (Subic), which then completes the circuit by exciting EC neurons. For simplicity, only feedforward excitatory projections of the classic trisynaptic pathway are depicted. Omitted are backward projections and local circuit interactions. *Note:* +, excitatory projection; −, inhibitory projection.

## ION CHANNELS

Neuronal excitation depends on the number, type, and distribution of ion channels within the neuronal membrane. Two major types of ion channels are responsible for the inhibitory and excitatory activity comprising normal neuronal function: voltage-gated channels and ligand-gated channels. Voltage-gated sodium and calcium channels depolarize the cell membrane toward the action potential threshold. Voltage-gated potassium channels largely dampen neuronal excitation by repolarizing the membrane potential after an action potential or by opposing depolarizing conductances to keep the membrane potential below threshold. A variety of ion conductances operative in the subthreshold voltage range also sculpt neuronal activity. Voltage-gated channels are activated by membrane potential changes, which subsequently alter the conformational state of the channel and allow selective passage of charged ions through a pore.

Ligand-gated receptors include those mediating excitation (glutamate receptors) and inhibition ( $\gamma$ -aminobutyric acid, or GABA, receptors). A neurotransmitter (prepackaged in vesicles) is released from a presynaptic terminal (following presynaptic depolarization and calcium influx) into the synaptic cleft; the neurotransmitter then binds with selective affinity to a membrane-bound receptor on the postsynaptic membrane. Binding of a neurotransmitter to its receptor activates a cascade of events, including a conformational shift to reveal an ion-permeant pore. Passage of ions across these channels results in either depolarization (e.g., inward flux of cations) or hyperpolarization (e.g., inward flux of anions or outward flux of cations). Excitability can also be modified posttranslationally, for example, by receptor phosphorylation or by second-messenger pathways and modified gene expression.

## VOLTAGE-DEPENDENT MEMBRANE CONDUCTANCES

### Depolarizing (Excitatory) Conductances

A rapidly inactivating inward sodium conductance underlies the depolarizing phase of the action potential, and a non-inactivating, persistent sodium current can augment cell depolarization (e.g., produced by excitatory synaptic input) in the voltage range immediately subthreshold for spike initiation (Crill, 1996; Hille, 2001). Dysfunction of either the rapidly inactivating sodium current or persistent sodium current can alter neuronal excitability and enhance the propensity for epileptic firing (George, 2005; Stafstrom, 2007b).

Sodium channels consist of a complex of three polypeptide subunits; a major  $\alpha$ -subunit forms the channel pore, and two smaller  $\beta$ -subunits influence the assembly and kinetic properties of the  $\alpha$ -subunit. The shape of the action potentials is determined by the types of  $\alpha$ - and  $\beta$ -subunits present in an individual neuron (Catterall et al., 2005). Many anticonvulsants act in part through interactions with voltage-dependent sodium channels, including phenytoin, carbamazepine, oxcarbazepine, felbamate, and lamotrigine (Rogawski and Löscher, 2004).

Neurons also display voltage-gated inward calcium conductances. In the hippocampus, prominent calcium currents occur in CA3 pyramidal cells, especially in dendrites, and underlie burst discharges in these cells (Wong and Prince, 1978). Activation of voltage-dependent calcium channels contributes to the depolarizing phase of the action potential and can affect neurotransmitter release, gene expression, and neuronal firing patterns. There are several distinct subtypes of calcium channels, distinguished on the basis of electrophysiological properties, pharmacological profile, molecular structure, and cellular localization (Catterall et al., 2003). The molecular structure of voltage-gated calcium channels is similar to that of sodium channels. Voltage-dependent calcium channels are hetero-oligomeric complexes comprised of a principal pore-forming  $\alpha_1$ -subunit and one or more smaller subunits ( $\alpha_2$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) that are not obligatory for normal activity but can modulate the kinetic properties of the channel.



## Hyperpolarizing (Inhibitory) Conductances

Depolarizing sodium and calcium currents are counterbalanced by an array of voltage-dependent hyperpolarizing currents, primarily via potassium channels. Potassium channels represent the largest and most diverse family of voltage-gated ionic channels and function to inhibit or decrease excitation in the nervous system (Hille, 2001). The prototypic voltage-gated potassium channel is composed of four membrane-spanning  $\alpha$ -subunits and four regulatory  $\beta$ -subunits, which are assembled in an octameric complex to form an ion-selective pore (Gutman et al., 2005). In hippocampal neurons, potassium conductances include: (1) a leak conductance, which is a major determinant of the resting membrane potential; (2) an inward rectifier (involving the flux of other ions), which is activated by hyperpolarization; (3) a large set of delayed rectifiers, which are involved in the termination of action potentials and repolarization of the neuron's membrane potential; (4) an A-current, which helps determine inter-spike intervals and thus affects the rate of cell firing; (5) an M-current, which is sensitive to cholinergic muscarinic agonists and affects the resting membrane potential and rate of cell firing; and (6) a family of calcium-activated potassium conductances that are sensitive to intracellular calcium concentration and affect the cell firing rate and interburst interval.

Although facilitation of these hyperpolarizing conductances could be viewed as potentially anticonvulsant, none of the traditional anticonvulsants is thought to act directly and principally on voltage-gated potassium channels. By contrast, newer anticonvulsants appear to act in part by affecting potassium channel function (Rogawski, 2000; Wickenden, 2002); for example, topiramate induces a steady membrane hyperpolarization mediated by a potassium conductance (Herrero et al., 2002), and levetiracetam blocks sustained repetitive firing by paradoxically decreasing voltage-gated potassium currents (Madeja et al., 2003). Retigabine, an investigational compound with broad efficacy in animal seizure models, appears to enhance activation of KCNQ2 and KCNQ3 potassium channels (members of the so-called Kv7 family), thus increasing the effectiveness of the M-type potassium current, which acts as a brake on repetitive neuronal firing (Miceli et al., 2008; Rogawski and Bazil, 2008). This is a particularly intriguing finding given that mutations in genes encoding these proteins have been linked to a rare form of inherited epilepsy, benign familial neonatal convulsions (Biervet et al., 1998).

## SYNAPTIC PHYSIOLOGY

### Inhibitory Synaptic Transmission

Synaptic inhibition in the hippocampus is mediated by two basic circuit configurations: (1) Feedback or recurrent inhibition occurs when excitatory principal neurons synapse with and excite inhibitory interneurons, which, in turn, project back to the principal neurons and inhibit them (i.e., a negative-feedback loop). (2) Feedforward inhibition occurs when axons projecting into the region synapse with and directly activate inhibitory interneurons, which then inhibit principal neurons. Both feedforward and feedback inhibitory circuits abound in the hippocampal formation and utilize GABA as the neurotransmitter.

GABA, the principal inhibitory neurotransmitter in the mammalian central nervous system, is a neutral amino acid synthesized from glutamic acid by the rate-limiting enzyme glutamic acid decarboxylase (GAD). GAD requires pyridoxine (vitamin B<sub>6</sub>) as a cofactor; inherited deficiency of pyridoxine or diminished responsiveness to pyridoxine is a cause of intractable neonatal seizures (Gospe, 2006). GABA, released from axon terminals, binds to at least two classes of receptors—GABA<sub>A</sub> and GABA<sub>B</sub>—which are found on almost all cortical neurons (Martin and Olsen, 2000). In addition, GABA<sub>A</sub> receptors are found on glia, where they may exert a role in the regulation of excitability (Sierra-Paredes and Sierra-Marcuño, 2007).

The GABA<sub>A</sub> receptor is a macromolecular receptor complex consisting of an ion pore as well as binding sites for agonists and a variety of allosteric modulators, such as benzodiazepines and barbiturates, each differentially affecting the kinetic properties of the receptor (Olsen and Sieghart,

2008). The GABA<sub>A</sub> receptor is a heteropentameric complex composed of combinations of several polypeptide subunits arranged in topographical fashion to form an ion channel. This channel is selectively permeable to chloride (and bicarbonate) ions. To date, seven different subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\pi$ ,  $\rho$ ) have been described, each with one or more subtypes. Although several thousand receptor isoforms are possible from differential expression and assembly of these various subtypes, there is likely to be only a limited number of functional combinations, but the precise subunit composition of native GABA<sub>A</sub> receptors has yet to be identified. Most functional GABA<sub>A</sub> receptors follow the general motif of containing either  $\alpha$  and  $\beta$  or  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits with uncertain stoichiometry. Because individual subunits might be differentially sensitive to pharmacological agents, GABA receptor subunits represent potentially useful molecular targets for new anticonvulsants.

Activation of GABA<sub>A</sub> receptors on the somata of mature cortical neurons generally results in the influx of Cl<sup>-</sup> and consequent membrane hyperpolarization, thus inhibiting cell discharge. In hippocampal cell dendrites and in the immature brain, however, GABA<sub>A</sub> receptor activation causes *depolarization* of the postsynaptic membrane. This reversal of the conventional GABA<sub>A</sub> effect is thought to reflect a reversed Cl<sup>-</sup> electrochemical gradient, a consequence of the immature expression of the K<sup>+</sup>-Cl<sup>-</sup> cotransporter KCC2, which ordinarily renders GABA hyperpolarizing (Rivera et al., 1999; Staley, 2006).

In addition to GABA<sub>A</sub> receptors, “metabotropic” GABA<sub>B</sub> receptors are located on both postsynaptic membrane and on presynaptic terminals. Metabotropic receptors do not form an ion channel pore; instead, GABA<sub>B</sub> receptors act through GTP-binding proteins to control calcium or potassium conductances. Whereas GABA<sub>A</sub> receptors generate fast high-conductance inhibitory postsynaptic potentials (IPSPs) close to the cell body, GABA<sub>B</sub> receptors on the postsynaptic membrane mediate slow, long-lasting, low-conductance IPSPs, primarily in hippocampal pyramidal cell dendrites. Perhaps of greater functional significance, activation of GABA<sub>B</sub> receptors on axon terminals blocks synaptic release of neurotransmitter. It is thought that GABA<sub>B</sub> receptors are associated with terminals that release GABA onto postsynaptic GABA<sub>A</sub> receptors. In such cases, activation of GABA<sub>B</sub> receptors reduces the amount of GABA released, resulting in disinhibition (Simeone et al., 2003).

### Excitatory Synaptic Transmission

Glutamate, an excitatory amino acid, is the principal excitatory neurotransmitter of the mammalian central nervous system. Glutamatergic pathways are widespread throughout the brain, and excitatory amino acid activity is critical to normal brain development and activity-dependent synaptic plasticity (Simeone et al., 2004). There are two classes of glutamate receptors: ionotropic and metabotropic. Ionotropic glutamate receptors are broadly divided into *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, based on biophysical properties and pharmacological profiles (Dingledine et al., 1999). Each subtype of glutamate receptor consists of a multimeric assembly of subunits that determine its distinct functional properties. Glutamate receptor channel subunits are currently classified into six subfamilies based on amino acid sequence homology.

The NMDA receptor contains a binding site for glutamate (or NMDA) and a recognition site for a variety of modulators (e.g., glycine, polyamines, MK-801, zinc). NMDA receptors also display voltage-dependent block by magnesium ions (Collingridge et al., 1988). When the membrane is depolarized and the magnesium block of the NMDA receptor is alleviated, activation of the NMDA receptor results in an influx of calcium and sodium ions. Calcium entry is central to the initiation of a number of second-messenger pathways—for example, stimulation of a variety of kinases that subsequently activate signal transduction cascades leading to changes in transcriptional regulation. Activation of the NMDA receptor leads to generation of relatively slow and long-lasting excitatory postsynaptic potentials (EPSPs). These synaptic events contribute to epileptiform burst discharges, and NMDA receptor blockade results in the attenuation of bursting activity in many models of epileptiform activity (Gean, 1990; Kalia et al., 2008).

Non-NMDA ionotropic receptors are divided into  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (Dingledine et al., 1999). The AMPA receptor is responsible for the major part of the EPSP—fast-rising and brief in duration—generated by the release of glutamate onto postsynaptic neurons. In addition, the depolarization generated via AMPA receptors is necessary for effective activation of NMDA receptors. Consequently, AMPA receptor antagonists block most excitatory synaptic activity in pyramidal neurons.

Metabotropic glutamate receptors represent a large, heterogeneous family of G-protein-coupled receptors, which subsequently activate various transduction pathways, such as phosphoinositide hydrolysis and activation of adenylate cyclase and phospholipases C and D (Conn, 2003). These metabotropic receptors are important modulators of voltage-dependent potassium and calcium channels, nonselective cation currents, and ligand-gated receptors (i.e., GABA and glutamate receptors) and can regulate glutamate release. Hence, it is not surprising that they have been invoked in a wide variety of neurological processes (e.g., long-term potentiation, or LTP) and disease states (including epilepsy) (Ure et al., 2006; Wong et al., 2004). Different metabotropic glutamate receptor subtypes are specific for different intracellular processes. Although ubiquitous within the central nervous system, subtypes of metabotropic receptors appear to be differentially localized.

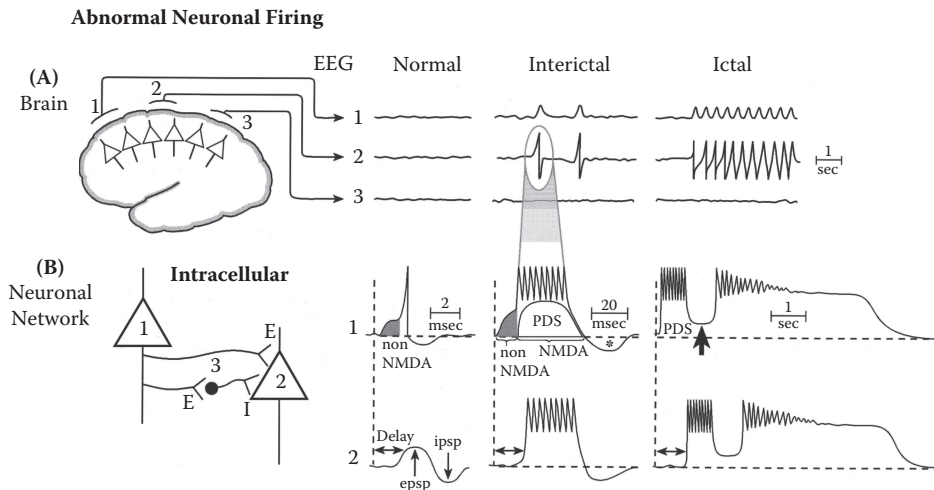
## PATHOPHYSIOLOGY OF EPILEPTIC FIRING

### ABNORMAL NEURONAL FIRING

At the neuronal cellular and network levels, there has been a concerted effort, extending over several decades, to understand the mechanisms governing the transition from normal firing to interictal epileptiform bursts to an ictal state, as well as the evolution of electrophysiological changes that terminate a seizure and underlie postictal changes (Lado and Moshe, 2008; Stafstrom, 2004). Likewise, mechanisms underlying epileptogenesis, the transition from normal brain to epileptic brain, represents both a critical knowledge gap and an opportunity for selective therapeutics (Clark and Wilson, 1999; Dichter, 2009; Dudek and Sutula, 2007). Understanding the scientific basis of both seizure generation and epileptogenesis requires correlated laboratory and clinical investigations. Much of our understanding of epilepsy mechanisms comes from *in vivo* animal models and *in vitro* electrophysiological studies.

As discussed earlier, consideration of the imbalance between excitatory and inhibitory factors helps to guide the approach to the mechanisms involved. Mutations have been identified in genes coding for ion channel proteins in both humans and animal models, many of which express seizures as a phenotype. These so-called epilepsy “channelopathies” represent a window for dissecting mechanisms of seizure genesis (Helbig et al., 2008; Reid et al., 2009). In addition, mutations in genes coding for proteins responsible for neurotransmitter transport and biogenesis, as well as receptor trafficking to the correct membrane location, are expanding the repertoire of epilepsy mechanisms (Hirose, 2006; Macdonald and Kang, 2009).

Figure 1.3 depicts EEG and intracellular changes that can be seen in normal, interictal, and ictal states. In the normal situation, action potentials (which represent “all-or-none” events) are generated when the neuronal membrane potential reaches the threshold for firing (approximately  $-40$  mV). These discharges may influence the activity of adjacent neurons through electrical field (i.e., ephaptic) or synaptic mechanisms, resulting in an EPSP. Nearby inhibitory interneurons may also be activated, after a brief delay, giving rise to an IPSP. The activity recorded in the target neuron (neuron 2 of Figure 1.3B) will reflect the temporal and spatial summation of both EPSP and IPSP inputs. When extrapolated to multiple synaptic contacts, the sculpting of individual cellular responses modulated by various degrees of inhibition can be envisioned. Further, when considering that a single inhibitory interneuron can connect with hundreds or thousands of pyramidal



**FIGURE 1.3** Abnormal neuronal firing at the levels of (A) the brain and (B) a simplified neuronal network, consisting of two excitatory neurons (1 and 2) and an inhibitory interneuron (filled black circle, 3). EEG (top set of traces) and intracellular recordings (bottom set of traces) are shown for the normal (left column), interictal (middle column), and ictal (right column) conditions. Numbered traces refer to like-numbered recording sites. Note time-scale differences in different traces. (A) Three EEG electrodes record activity from superficial neocortical neurons. In the normal case, activity is low voltage and desynchronized (neurons are not firing together in synchrony). In the interictal condition, large spikes are seen focally at electrode 2 (and to a lesser extent at electrode 1, where they might be termed “sharp waves”), representing synchronized firing of a large population of hyperexcitable neurons (expanded in time below). The ictal state is characterized by a long run of spikes. (B) At the neuronal network level, the intracellular correlate of the interictal EEG spike is the paroxysmal depolarization shift (PDS). The PDS is initiated by a non-NMDA-mediated fast EPSP (shaded area) but is maintained by a longer, larger NMDA-mediated EPSP. The post-PDS hyperpolarization (asterisk) temporarily stabilizes the neuron. If this post-PDS hyperpolarization fails (right column, thick arrow), ictal discharge can occur. The lowermost traces, recordings from neuron 2, show activity similar to that recorded in neuron 1, with some delay (double-headed horizontal arrow). Activation of inhibitory neuron 3 by the firing of neuron 1 prevents neuron 2 from generating an action potential (the IPSP counters the depolarization caused by the EPSP). If neuron 2 does reach the firing threshold, additional neurons will be recruited, leading to an entire network firing in synchrony (seizure). (From Stafstrom, C.E., in *Epilepsy and the Ketogenic Diet*, Stafstrom, C.E. and Rho, J.M., Eds., Humana Press, Totowa, NJ, 2004, p. 3. With permission.)

neurons, it is straightforward to see how hypersynchronous behavior can be influenced by even a single cell. For localized hyperexcitability to spread to adjacent areas, the epileptic firing must overcome the powerful inhibitory influences that normally keep aberrant excitability in check (i.e., the “inhibitory surround”).

### PAROXYSMAL DEPOLARIZATION SHIFT

The intracellular correlate of the focal interictal epileptiform discharge on EEG is known as the paroxysmal depolarization shift (PDS) (Ayala, 1983). The PDS is seen when recording changes in the membrane potential of a single neuron with a microelectrode while simultaneously recording a focal spike on EEG (Figure 1.3B). Initially, there is a rapid shift in the membrane potential in a depolarizing direction initiated by synaptic forces, followed by a burst of repetitive action potentials on a depolarizing plateau potential lasting several hundred milliseconds (Johnston and Brown, 1984). The initial depolarization is mediated by non-NMDA glutamate receptors (i.e., AMPA receptors), while the sustained depolarization is a consequence of NMDA receptor activation. Afterward,