T. Sutula and A. Pitkänen (Eds.)
Progress in Brain Research, Vol. 135

CHAPTER 2

Are seizures harmful: what can we learn from animal models?

Andrew J. Cole *, Sookyong Koh and Yi Zheng

Epilepsy Research Laboratory, Massachusetts General Hospital and Department of Neurology, Harvard Medical School, Boston, MA 02114, USA

Abstract: Epilepsy is a brain disease that requires distributed neuronal networks for its expression. Several characteristics of epilepsy, including its natural history, the latency between an initial insult and the first manifestation of seizures, the complex interaction of seizures with development as a function of developmental stage, the modulating effect of systemic physiological responses, and the fact that seizures are ultimately defined by a combination of electrical and behavioral criteria all suggest that epilepsy should ideally be studied in an intact whole animal preparation. Such preparations offer the ability to study acute and chronic changes in brain structure and function after single or repeated seizures. Animal models have major limitations, however, including strain specificity, difficulty in isolating potentially confounding variables, a relative lack of accessible higher cortical functions, such as language and abstract processing, and shorter lifespans that may be insufficient to allow the complete expression of seizure-related injury. Information we have learned from animal studies includes a broad understanding of the chemical, molecular and anatomic consequences of seizures, including their temporal and spatial relationships to each other, and information on the consequences of seizures as a function of development. Recent studies have cast light on potential mechanisms of resistance to seizure-induced injury in the developing brain. In the future, we can anticipate that animal models will continue to be useful, especially when whole-animal preparations are used to generate material for detailed in vitro examination.

Introduction

Epilepsy is a brain disease, and epileptic seizures result from abnormal paroxysmal activity of populations of neurons. The careful reader will note that by definition epilepsy and epileptic seizures cannot be conceptualized as disorders of single neurons per se. It is perhaps less clear whether the disease state and its primary symptom can be successfully recapitulated in an isolated array of neurons, either a plate of cultured cells or an ex vivo preparation, such as a hippocampal or cortical slice. Furthermore, epilepsy, a disorder manifest by recurrent unprovoked seizures, is by definition a chronic disease, or at least a disease that manifests itself over a period of time, not simply at an instant. Reductionist models typically are acute, in the case of ex vivo preparations, or developmentally disturbed, in the case of cultured cells. Moreover, epilepsy occurs in the context of ongoing physiological processes such as perfusion, oxygenation, glucose metabolism, acid-base regulation, thermoregulation, endocrine modulation and the like, some of which may have critical interactions with the disease state itself. While the confounding influences of normal physiological responses may make the interpretation of whole animal experiments difficult, their absence may represent an important and under-recognized limitation of reductionist approaches. For these reasons, it seems that

* Correspondence to: A.J. Cole, MGH Epilepsy Service, VBK 830, Massachusetts General Hospital, Fruit Street, Boston, MA 02114, USA. Tel.: +1-617-726-3311; Fax: +1-617-726-9250;
E-mail: cole.andrew@mgh.harvard.edu

TABLE 1
The spectrum of animal models

Electrical stimulation	Maximal electroconvulsive seizures (MECS)	
	Perforant-path stimulation (PPS)	
	Kindling	
Chemoconvulsants	Systemic	Kainate
		Pilocarpine
		Picrotoxin
		Bicuculline
		Penicillin
	Intracerebral	Kainate
		Pilocarpine
		Picrotoxin
		Bicuculline
		Tetanus toxin
		Pertussis toxin
	Topical	Alumina cream
		Penicillin
Physical models	Hyperthermia	
	Freeze lesions	
	Photic stimulation (*Papio papio*)	
	Auditory stimulation (Swiss DBA2 mice)	
Genetic models	Spontaneous	Genetically epilepsy-prone rat strain (GEPRS)
		Strasbourg rats (Absence)
		Epileptic beagles
	Mutant	Stargazer
		Lurcher
		Totterer
		Mocha
	Transgenic	
	Knockout	
Spontaneous seizure models	Post-kindling	
	Post-kainate	
	Post-pilocarpine	

the most effective way to model epilepsy should be to utilize whole animal preparations. And indeed a considerable body of epilepsy research work has utilized animal models, leading to many fundamental insights. In this chapter, we will review some of the important uses of animal models, discuss their limitations, and consider the role of animal models going forward. In particular, we will focus on the issue of whether a single or initial seizure is harmful, and how it might relate to the development of epilepsy.

The spectrum of animal models

A wide variety of animal models of epilepsy and epileptic seizures exist. The major models are listed in Table 1. The tremendous diversity of available animal models offers both opportunity and challenge. Many of the models are acute, and many are, in fact, models of status epilepticus which may have important differences from isolated seizures. Genetic models often have phenotypes that are complex, with seizures as only one manifestation of a more pervasive structural, functional or developmental problem. Strain differences in responses to epileptogenic or convulsant stimuli make comparisons between animals from different laboratories difficult, but may offer opportunity to identify pro- or anti-epileptic genes using differential screening approaches (Schauwecker and Steward, 1997; Sandberg et al., 2000; Schauwecker, 2000). Chemo-

TABLE 2

Advantages and limitations of animal models

Advantages

- Allow assessment of seizure causes and consequences in an intact preparation
- Survival time can be adjusted to examine temporal evolution of post-ictal changes
- Developmental stage can be selected, and events at one point in development can be studied with respect to effects manifested at a later stage
- Repeated events can studied (e.g. kindling)
- Effects of physiological milieu are integrated

Limitations

- Many offer snapshot picture of ictal/post-ictal events
- Many result in status epilepticus, which may not adequately model typical epilepsy
- Strain differences in seizures and responses highlight the difficulties in generalizing findings in a specific model
- Effects of physiological milieu are integrated

TABLE 3

Situations in which whole-animal models are required

- To examine transduction of an input function into an output function without requiring knowledge of the mechanism
- To examine systems' level physiology where distant or unknown connections may have a role
- To study anatomic patterns of responses
- To study the relationship of anatomic findings, e.g. injury, plasticity to specific molecular markers
- To study developmentally regulated anatomic, biochemical and functional events
- To study the effects of chronic or recurrent seizures
- To study the effects of specific genetic manipulations on phenotype
- To examine the influence of physiological milieu on seizures and their consequences
- To generate biological material for examination after seizures
- To confirm findings from reductionist systems in the scaled-up whole animal situation

convulsants may have systemic effects that are either completely independent from seizures, or even more problematic, result in seizures only as a secondary consequence to injury or functional disturbance. Finally, some of the important correlates of seizures and epilepsy, such as memory loss, behavioral changes, and secondary psychiatric disturbance are at best difficult to measure in epileptic animals. By contrast, all animal models share the property of having anatomically intact central nervous systems with functional connections and measurable efferent responses. Animals can be developmentally monitored, and prolonged survival is possible to allow examination of delayed effects of specific stimuli or treatment. Most recently, the ability to manipulate the genetic endowment of model animals has opened the door to allowing examination of complex interactions between genes and behavior, between nature and nurture. The advantages and disadvantages of animal models are listed in Table 2.

In the remainder of this chapter, we will identify specific situations where animal models should be particularly useful, and to offer examples of insights that have come from animal models that might not have been available elsewhere. Several situations in which animal models have an obvious utility can be defined and are listed in Table 3.

Insights from animal models

Seizures trigger a cascade of biochemical, anatomic and functional changes in the central nervous system

It has long been recognized that critical aspects of brain development are activity-dependent. For example, the pioneering experiments of Hubel and Weisel established the critical role of visual input in the post-natal organization of the visual system, including the establishment of cortical columns, pruning of redundant connections, and establishment of the critical property of surround inhibition (Hubel and Wiesel, 1970; Hubel et al., 1977). Later studies have demonstrated that cortical plasticity, both functional and anatomic, occur in response to altered afferent activity, e.g. amputation or fusion of digits in primates (Merzenich et al., 1984; Allard et al., 1991). Perhaps surprisingly, our appreciation that brief seizures trigger long-term changes in CNS properties is relatively recent. Fig. 1A summarizes, in a schematic form, some of the events that occur after seizures. A critical point is that each of these observations came from animal studies. As an example, consider the observation that brief seizures induced by pentylenetetrazole or maximal electroconvulsive treatment results in rapid and transient expression of a large class of immediate early genes, many encoding transcription factors. While cell culture studies of

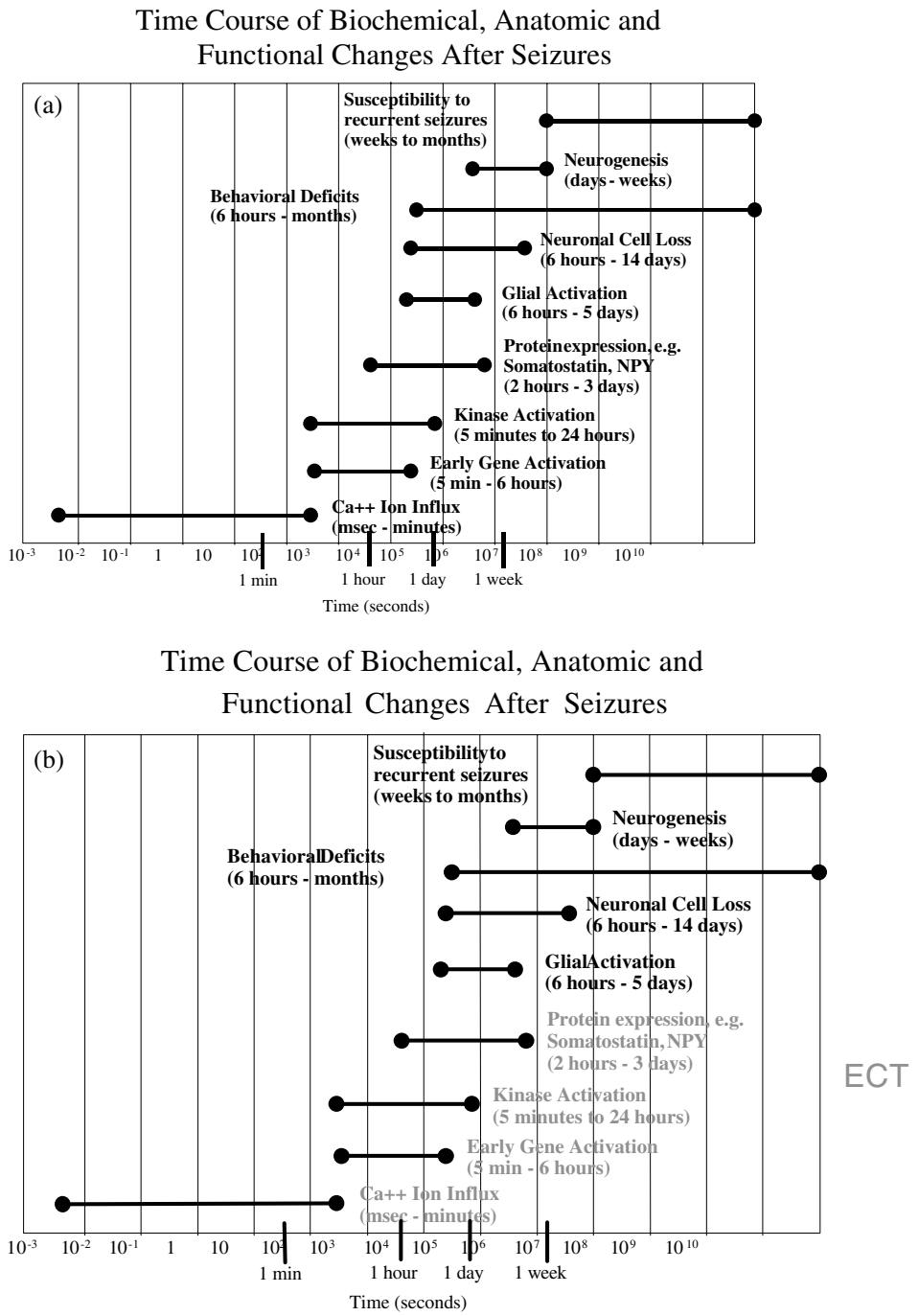

Fig. 1. (A) Diagrammatic representation of some of the biochemical, anatomic and functional changes seen after seizures in a variety of electrical and chemoconvulsant animal models. (B) Diagrammatic representation of changes seen in ECT models (indicated in green). Note that many of the lasting changes have not been reported after single or repeated ECT.

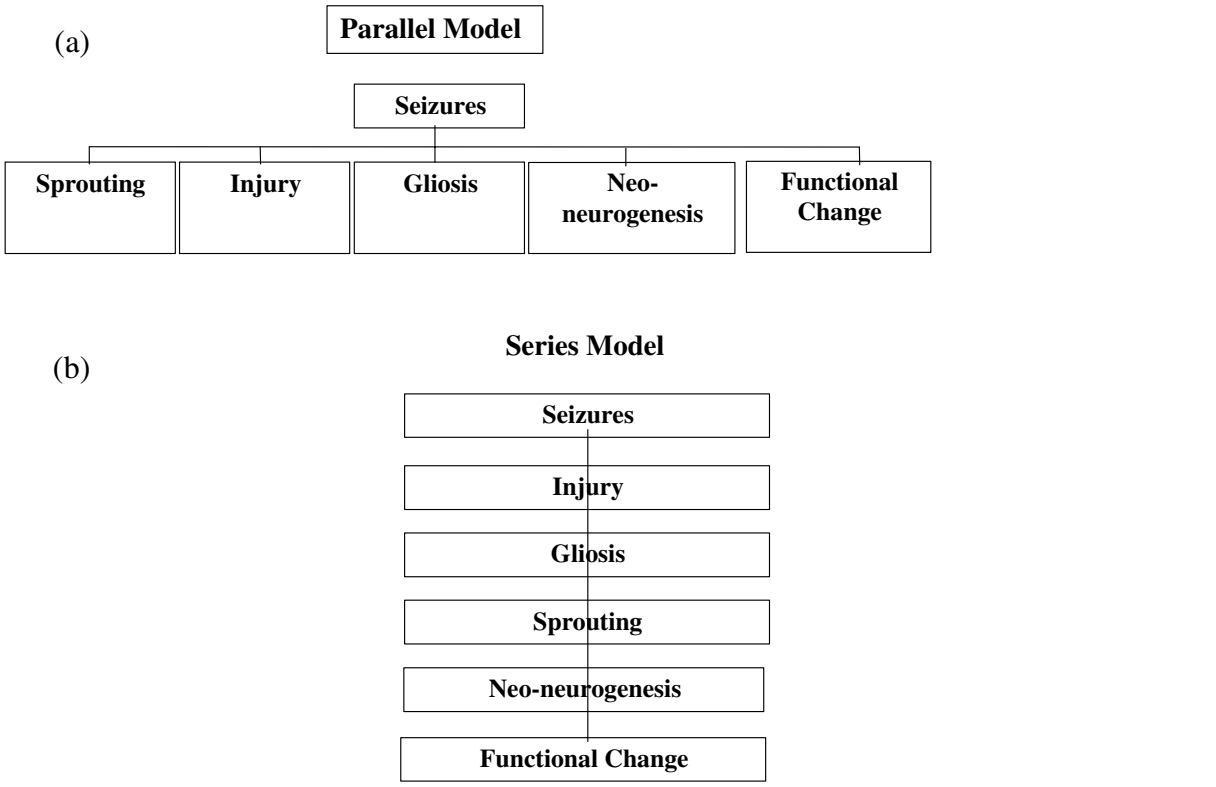

Fig. 2. Theoretical representation of the relationship between various documented changes occurring after seizures. (A) Parallel model indicates that specific consequences of seizures may occur independently of each other. (B) Series model represents the alternative hypothesis that later latency changes depend on earlier events for their expression.

activity-dependent gene expression could have suggested this phenomenon, only whole animal studies allowed us to appreciate the extraordinary anatomic specificity of this response (Morgan et al., 1987; Saffen et al., 1988; Cole et al., 1989). Similarly, observation of a variety of events including kinase activation (Murray et al., 1998, 1999; Anderson et al., 2000), neuropeptide regulation (Gall et al., 1990; Baraban et al., 1993; Vezzani et al., 1999; Madsen et al., 2000), cell loss (Margerison and Corsellis, 1966; Corsellis and Meldrum, 1976; Schwob et al., 1980; Gloor, 1991; Sloviter, 1994), mossy fiber sprouting (Sutula et al., 1988; Cavazos et al., 1991; Wuarin and Dudek, 1996; Patrylo et al., 1999), enhanced neoneurogenesis (Parent and Lowenstein, 1994), altered receptor expression, chronic behavioral deficits, and altered susceptibility to recurrent seizures are all the result of animal studies. With the exception of the latter phenomenon (see next section), it remains unclear whether any or all of these effects of a single seizure contribute to the development of epilepsy. Moreover, it is unclear whether whatever contribution they may have is organized in series or in parallel (Fig. 2). Obviously this issue has critical therapeutic implications.

Early life seizures increase susceptibility to later life seizures and neuronal injury

An important observation from animal studies is that seizures early in life result in a long-term susceptibility to recurrent seizures with resultant neuronal injury and behavioral deficits later in life. This finding has been established in multiple laboratories using a variety of animal models including repeated kainate administration (Koh et al., 1999), early life hyperthermic seizures (Dube et al., 2000), and early-life fluroythyl-induced status (Holmes et al., 1998;

Schmid et al., 1999). Each of these models shares the property that anatomic injury is difficult or impossible to detect after the initial insult, suggesting that the resulting susceptibility is the consequence of a functional change in network properties.

These studies raise several important questions:

(1) What is the transduction process that results in enhanced seizure-susceptibility later in life?
(2) Why are juvenile animals resistant to seizure-induced injury?
(3) Is there a critical point in development before or after which seizures no longer have this long-term effect? [1]

Early-life seizures alter synaptic connectivity in developing brain

One hypothesis, unproven, is that early life seizures may stabilize immature synaptic connections normally destined for removal, thereby resulting in an intrinsically hyperexcitable brain in adulthood. Evidence for this concept comes from Grigonis and Murphy (1994) who showed that topical application of penicillin to immature rabbit visual cortex resulted in persistence of the immature pattern of callosal projections without the pruning that occurs in normal development. By contrast, Swann and colleagues have found that early seizures resulted in a reduction in dendritic spine density, suggesting an alternative substrate for lasting functional change (Jiang et al., 1998). Similar findings have been reported in human surgical tissue (Multani et al., 1994) and in chronic animal seizure models (Willmore et al., 1980) as well.

Resistance to neuronal injury in the juvenile brain

Many studies have established that in the rodent, seizures prior to P21 result in little, if any, detectable injury. In the adult brain, we have observed that treatment with nerve growth factor attenuates hippocampal injury after kainate-induced seizures (Weiss et al., 1995). Ambient levels of NGF are maximal during development, peaking at P14–15. We therefore hypothesized that high ambient levels of NGF may prevent seizure-induced neuronal injury in the immature brain. To test this hypothesis, we selectively lesioned cholinergic neurons bearing the low-affinity neurotrophin receptor, p^{75ntfr} using a selective immunotoxin, 192-IgG-saporin. In preliminary experiments, rats treated with 192-IgG-saporin on P7 that showed complete loss of basal forebrain cholinergic neurons and marked depletion of acetylcholinesterase stained terminals in hippocampus also demonstrated severe selective cell loss in CA3 after subsequent treatment with kainate on P16. By contrast, animals treated with saline on P7 and animals in which the saporin treatment was unsuccessful in depleting cholinergic neurons had no kainate-induced hippocampal injury (Fig. 3). While these experiments are consistent with our hypothesis, because p^{75ntfr} binds several neurotrophins including BDNF, NT-3 and NT-4/5 it remains unclear whether high levels of NGF are critical to neuronal survival in this experimental paradigm. Moreover, in light of the fact that seizures induce NGF expression, and the findings of Grigonis and Murphy described above, we must consider the possibility that enhanced neuronal survival may have negative consequences with respect to later seizure susceptibility.

Are seizures neuroprotective?

The preceding discussion emphasizes the commonly held belief that seizures are harmful. Seizure-induced phenomena, such as neuronal loss, synaptic remodeling and aberrant neuronal proliferation, especially in the context of decreased performance on behavioral testing and enhanced susceptibility to recurrent seizures would seem to support this notion. Long clinical experience and recent experimental data, however, have challenged this axiom. For example, for many years psychiatrists have been treating patients with refractory depression with electroconvulsive seizures, often with gratifying results. There is little to suggest that ECT, as typically applied causes significant anatomic injury or behavioral dysfunction, although patients who receive hundreds of treatments may provide anecdotal exceptions. Suggestions that ECT causes progressive brain atrophy and hippocampal changes have not been supported by careful clinical studies (Sheline et al., 1999; Ende

[1] Because we have no data on this point, this question will not be discussed.

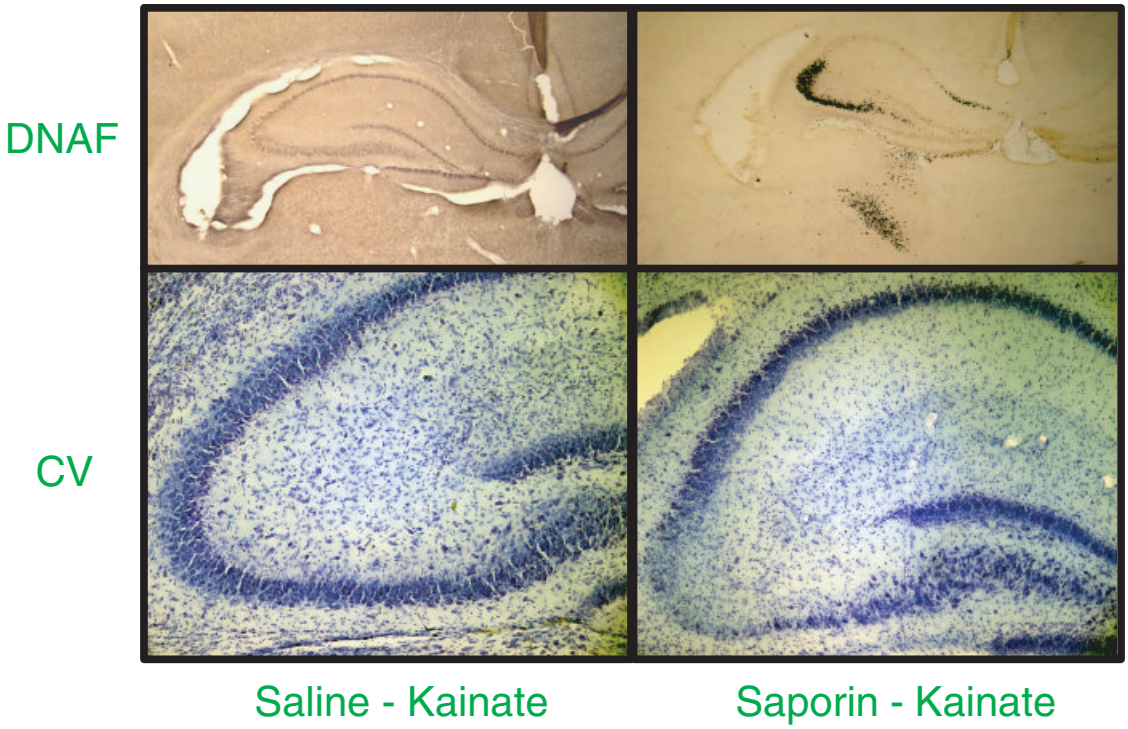

Fig. 3. Neuronal injury after kainate-induced seizures in P15 rats. DNAF indicates DNA fragmentation, a marker of neuronal injury. CV indicates Cresyl violet Nissl staining showing neuronal integrity. Saline–Kainate indicates animals pretreated with intraventricular saline on P7, prior to kainate on P15. Saporin–Kainate indicates animal treated with 192-IgG-Saporin intraventricularly on P7 prior to kainate on P15. Note injury and cell loss in CA3 after saporin treatment.

et al., 2000) which have concluded that depression itself and its pharmacological treatment are confounding variables with a more powerful effect on structure and function. By contrast, animal studies during development have pointed out injurious effects of ECT (Wasterlain and Plum, 1973; Jorgensen et al., 1980) and in experimental system electroconvulsive seizures have been shown to induce abnormal gene expression (Morgan et al., 1987; Saffen et al., 1988; Cole et al., 1990, 1997), kinase activation (Baraban et al., 1993), protein synthesis (Cole et al., 1990; Gall et al., 1991; Bhat et al., 1993), and neurotransmitter receptor expression (Bergstrom and Kellar, 1979; Kellar et al., 1981; Lerer, 1984; Green et al., 1986) (See Fig. 1B). These observations suggest that many of the early events occurring after seizures, while perhaps necessary, are not sufficient to mediate later emergence of neuronal injury, synaptic reorganization, and network dysfunction. They also emphasize the possibility that many of the events occurring after seizures are arranged in parallel, rather than in series (see Fig. 2A,B). Whether seizures are harmful, neutral or beneficial may depend on seizure type, e.g. brief electrically induced generalized attacks occurring in controlled clinical circumstances versus spontaneous seizures of variable duration occurring in an uncontrolled environment, or host characteristics, such as the presence of underlying neurological (as opposed to psychiatric) dysfunction or disease. In any case, these observations emphasize the need for cautious and unbiased interpretation of the observations gathered from experimental systems.

Recent experimental data have also challenged the notion that seizures are harmful. For example, Greenberg and colleagues (Sasahira et al., 1995) found that repeated bicuculline seizures separated by 1, 3, 5 or 7 days conferred a time-dependent protective effect against hippocampal injury induced by subsequent seizures in the CA3c sector of the hippocampus. They coined the term ‘epileptic tolerance’

to describe this phenomenon, and suggested that enhanced expression of heat-shock proteins caused by the initial seizure might be responsible for subsequent protection against recurrent seizure-induced injury. Their study suggested, however, that the protection was brief and of uncertain clinical relevance. McIntyre and colleagues (Kelly and McIntyre, 1994) have shown that kindling stimulation protects against kainate seizure-induced injury for up to 28 days, and Penner and colleagues have confirmed this result in a rapid kindling paradigm (Penner et al., 2001). Similarly, Gale and colleagues have reported in abstract form that repeated electroconvulsive seizures protect against kainate-seizure induced injury in experimental animals. ECT has been shown to cause declining seizure severity with repeated administration (Cole et al., 1990), perhaps the behavioral analogue of the relative refractory period described in synaptic physiology. It will therefore be important to review Gale's work critically to determine the duration of protection conferred.

?#1

Transgenic and knockout experiments

The ability to determine the genetic endowment of experimental animals has offered an important alternative to classical pharmacology for hypothesis testing. Rather than relying on specific agonists or antagonists, one can now directly block either the synthesis or activity of specific molecules and then examine the resultant phenotype. These studies may be confounded by at least two practical issues. First, animals born with altered genomes may utilize compensatory mechanisms to overcome the induced deficits, or they may develop abnormally in a manner that renders hypothesis testing impossible or irrelevant. The extreme example, of course, is the embryonic-lethal transgene or knockout. More subtle is the situation where alternative isoforms of specific gene products serve to partially or completely compensate for the genetic alteration. To some extent, the development of inducible expression systems for transgenes and conditional knockouts may partially overcome these limitations. Second, strain differences in genetic background may, in fact, have more influence on phenotype than the targeted genetic alteration itself. A graphic example of this phenomenon came from studies of p53 knockout animals. Several groups of investigators found that p53 knockout animals were protected against kainate seizure-induced neuronal injury (Morrison et al., 1996). Another group, however, using p53 knockout animals from a different source, were unable to reproduce that result (Schauwecker and Steward, 1997). It subsequently became apparent that the difference in the two studies was the result of strain differences in the genetic backgrounds of mice used to develop the knockout lines.

Animal models in the new millennium

As we move into the new millennium, a new trend in animal models is emerging that promises to offer powerful insights into the cause and effect of seizures. These models share the property that in vivo and in vitro techniques are combined to allow experiments that could not be conducted in either environment exclusively. While these strategies are perhaps best thought of as evolutionary, rather than revolutionary, they deserve special mention none the less. Three experimental paradigms provide illustrations of these approaches.

Use of biological material from genetically manipulated animals for in vitro study

Perhaps one of the most valuable uses of genetic manipulation results from the ability to harvest biological material from manipulated animals for in vitro studies. For example, recent studies have demonstrated that conditional knockout of the neuronal MAP kinase kinase (MEK) gene in hippocampus alters the characteristics of long-term potentiation as studied in hippocampal slices (Atkins et al., 1998; Selcher et al., 1999; Schafe et al., 2000) (Kelleher, personal communication). Similar approaches are now routinely undertaken to examine the effect of genetic manipulation on in vitro physiology using slice preparations and patch clamp techniques, and on neuronal viability and biochemical responses using primary neuronal cell culture techniques.

Receptor alterations in epileptic animals

Another example of the combination of in vitro and in vivo techniques comes from the work of

Coulter and colleagues. These investigators have developed spontaneously epileptic animals using the pilocarpine model. After documenting recurrent seizures, they have prepared hippocampal slices and documented physiological abnormalities, especially in the properties of GABA receptors (Gibbs et al., 1997). They have then gone on to use RT-PCR techniques on single cells from these slices to document changes in the specific GABA receptor subunits expressed in epileptic animals as compared to controls (Brooks-Kayal et al., 1998). Interestingly, an extension of this study to examine GABA receptor subunit expression in animals after the initial seizure, but before the development of spontaneous seizures could provide insight into the fundamental question before this meeting, whether a single seizure is harmful.

Microarray analysis of epileptic animals

A third example of the use of in vivo and in vitro techniques in combination illustrates the idea that this approach is really an evolutionary extension of older analytical methods where in situ techniques were used to characterize molecular responses to seizures. Microarray analysis of cDNAs generated from epileptic material offers an open-ended method for documenting altered gene expression that requires little de facto knowledge of the targets to be examined. In its earliest incarnations, 2-D gel electrophoresis of proteins and differential screening of cDNA libraries led to the identification of previously unknown molecular responses to abnormal activity. For example, the finding that Homer (Brakeman et al., 1997), GRIP (Dong et al., 1997) and ARC (Lyford et al., 1995) were regulated after seizures came from a differential screening strategy. Homer and GRIP are PDZ-domain containing proteins that interact with metabotropic- and AMPA-type glutamate receptors, respectively. ARC, by contrast, is expressed mainly in dendrites where it may mediate synaptic plasticity. Each of these proteins has the potential to mediate changes in synaptic efficiency that are long-lasting. More recently, this approach has been extended by the use of microarray analysis in which thousands of known and unknown transcripts can be quantitatively assessed in response to seizures and compared to controls to look for both up- and down-regulation of activity-dependent transcripts (Sandberg et al., 2000). Dingledine has used this approach to examine differences between early and late transcriptional responses, whereas Lowenstein and colleagues have concentrated on examining transcripts that are regulated both during development and after seizures. Applications of this technology, which starts with the whole animal and quickly moves to the in vitro environment will be limited only by the arrays of targets available and the imagination of investigators.

?#2

References

Allard, T., Clark, S.A., Jenkins, W.M. and Merzenich, M.M. (1991) Reorganization of somatosensory area 3b representations in adult owl monkeys after digital syndactyly. *J. Neurophysiol.*, 66(3): 1048–1058.

Anderson, A.E., Adams, J.P., Varga, A.W., Selcher, J.C., Feng, J., Trzaskos, J.M. and Sweatt, J.D. (2000) Essential role for ERK MAPK activation and K^+ channel phosphorylation in the kainate model of epilepsy. *Soc. Neurosci. Abstr.*, 26: 1836.

Atkins, C.M., Selcher, J.C., Petraitis, J.J., Trzaskos, J.M. and Sweatt, J.D. (1998) The MAPK cascade is required for mammalian associative learning. *Nat. Neurosci.*, 1(7): 602–609.

Baraban, J.M., Fiore, R., Sanghera, J.S., Paddon, H.B. and Pelech, S. (1993) Identification of p42 mitogen-activated protein kinase as a tyrosine kinase substrate activated by maximal electroconvulsive shock in hippocampus. *J. Neurochem.*, 60: 330–336.

Bergstrom, D.A. and Kellar, K.J. (1979) Effect of electroconvulsive shock on monoaminergic receptor binding sites in rat brain. *Nature*, 278: 464–466.

Bhat, R.V., Tausk, F.A., Baraban, J.M., Mains, R.E. and Eipper, B.A. (1993) Rapid increases in peptide processing enzyme expression in hippocampal neurons. *J. Neurochem.*, 61(4): 1315–1322.

Brakeman, P.R., Lanahan, A.A., O'Brien, R., Roche, K., Barnes, C.A., Huganir, R.L. and Worley, P.F. (1997) Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature*, 386(6622): 284–288.

Brooks-Kayal, A.R., Shumate, M.D., Jin, H., Rikhter, T.Y. and Coulter, D.A. (1998) Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat. Med.*, 4(10): 1166–1172.

Cavazos, J.E., Golarai, G. and Sutula, T.P. (1991) Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. *J. Neurosci.*, 11(9): 2795–2803.

Cole, A.J., Saffen, D.W., Baraban, J.M. and Worley, P.F. (1989) Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature*, 340: 474–476.

Cole, A.J., Abu Shakra, S., Saffen, D.W., Baraban, J.M. and Worley, P.F. (1990) Rapid rise in transcription factor mRNAs

in rat brain after electroshock-induced seizures. *J. Neurochem.*, 55: 1920–1927.

Cole, A.J., Koh, S., Liu, Z. and Holmes, G.L. (1997) DNA fragmentation in developing brain after kainate-induced seizures. *Epilepsia*, 38(Suppl. 8): 40.

Corsellis, J.A.N. and Meldrum, B.S. (1976) Epilepsy. In: W. Blackwood and J.A.N. Corsellis (Eds.), *Greenfield's Neuropathology*, 3 edn. Edward Arnold, London, pp. 771–795.

Dong, H., O'Brien, R.J., Fung, E.T., Lanahan, A.A., Worley, P.F. and Huganir, R.L. (1997) GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature*, 386(6622): 279–284.

Dube, C., Chen, K., Eghbal-Ahmadi, M., Brunson, K., Soltesz, I. and Baram, T.Z. (2000) Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. *Ann. Neurol.*, 47(3): 336–344.

Ende, G., Braus, D.F., Walter, S., Weber-Fahr, W. and Henn, F.A. (2000) The hippocampus in patients treated with electroconvulsive therapy: a proton magnetic resonance spectroscopic imaging study. *Arch. Gen. Psychiatry*, 57(10): 937–943.

Gall, C., Lauterborn, J., Isackson, P. and White, J. (1990) Seizures, neuropeptide regulation, and mRNA expression in the hippocampus. *Prog. Brain Res.*, 83: 371–390.

Gall, C., Lauterborn, J., Bundman, M., Murray, K. and Isackson, P.J. (1991) Seizures and the regulation of neurotrophic factor and neuropeptide gene expression in brain [Review]. *Epilepsy Res. Suppl.*, 4: 225–245.

Gibbs, J.W., Shumate, M.D. and Coulter, D.A. (1997) Differential epilepsy-associated alterations in postsynaptic GABA(A) receptor function in dentate granule and CA1 neurons. *J. Neurophysiol.*, 77(4): 1924–1938.

Gloor, P. (1991) Mesial temporal sclerosis: historical background and an overview from a modern perspective. In: H. Luders (Ed.), *Epilepsy Surgery*. Raven Press, New York, pp. 689–703.

Green, A.R., Heal, D.J. and Goodwin, G.M. (1986) The effects of electroconvulsive therapy and antidepressant drugs on monoamine receptors in rodent brain-similarities and differences. *Ciba Found. Symp.*, 123: 246–267.

Grigonis, A.M. and Murphy, E.H. (1994) The effects of epileptic cortical activity on the development of callosal projections. *Dev. Brain Res.*, 77(2): 251–255.

Holmes, G.L., Gairsa, J.L., Chevassus-Au-Louis, N. and Ben Ari, Y. (1998) Consequences of neonatal seizures in the rat: morphological and behavioral effects. *Ann. Neurol.*, 44(6): 845–857.

Hubel, D.H. and Wiesel, T.N. (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol.*, 206: 419–436.

Hubel, D.H., Wiesel, T.N. and LeVay, S. (1977) Plasticity of ocular dominance columns in monkey striate cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 278(961): 377–409.

Jiang, M., Lee, C.L., Smith, K.L. and Swann, J.W. (1998) Spine loss and other persistent alterations of hippocampal pyramidal cell dendrites in a model of early-onset epilepsy. *J. Neurosci.*, 18(20): 8356–8368.

Jorgensen, O.S., Dwyer, B. and Wasterlain, C.G. (1980) Synaptic proteins after electroconvulsive seizures in immature rats. *J. Neurochem.*, 35(5): 1235–1237.

Kellar, K.J., Cascio, C.S., Butler, J.A. and Kurtze, R.N. (1981) Differential effects of electroconvulsive shock and antidepressant drugs on serotonin-2 receptors in rat brain. *Eur. J. Pharmacol.*, 69: 515–518.

Kelly, M.E. and McIntyre, D.C. (1994) Hippocampal kindling protects several structures from the neuronal damage resulting from kainic acid-induced status epilepticus. *Brain Res.*, 634(2): 245–256.

Koh, S., Storey, T.W., Santos, T.C., Mian, A.Y. and Cole, A.J. (1999) Early-life seizures in rats increase susceptibility to seizure-induced brain injury in adulthood [In Process Citation]. *Neurology*, 53(5): 915–921.

Lerer, B. (1984) Electroconvulsive shock and neurotransmitter receptors: implications for mechanism of action and adverse effects of electroconvulsive therapy. *Biol. Psychiatry*, 19(3): 361–383.

Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A. and Worley, P.F. (1995) Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron*, 14(2): 433–445.

Madsen, T.M., Greisen, M.H., Nielsen, S.M., Bolwig, T.G. and Mikkelsen, J.D. (2000) Electroconvulsive stimuli enhance both neuropeptide Y receptor Y1 and Y2 messenger RNA expression and levels of binding in the rat hippocampus. *Neuroscience*, 98(1): 33–39.

Margerison, J.H. and Corsellis, J.A.N. (1966) Epilepsy and the temporal lobes: a clinical encephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain*, 89: 499–530.

Merzenich, M.M., Nelson, R.J., Stryker, M.P., Cynader, M.S., Schoppmann, A. and Zook, J.M. (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. *J. Comp. Neurol.*, 224(4): 591–605.

Morgan, J.I., Cohen, D.R., Hempstead, J.L. and Curran, T. (1987) Mapping patterns of c-fos expression in the central nervous system after seizure. *Science*, 237(4811): 192–197.

Morrison, R.S., Wenzel, H.J., Kinoshita, Y., Robbins, C.A., Donehower, L.A. and Schwartzkroin, P.A. (1996) Loss of the p53 tumor suppressor gene protects neurons from kainate-induced cell death. *J. Neurosci.*, 16(4): 1337–1345.

Multani, P., Myers, R.H., Blume, H.W., Schomer, D.L. and Sotrel, A. (1994) Neocortical dendritic pathology in human partial epilepsy: a quantitative Golgi study. *Epilepsia*, 35(4): 728–736.

Murray, B., Alessandrini, A., Cole, A.J., Yee, A.G. and Furshpan, E.J. (1998) Inhibition of the p44/42 MAP kinase pathway protects hippocampal neurons in a cell-culture model of seizure activity. *Proc. Natl. Acad. Sci. USA*, 95(20): 11975–11980.

Murray, B., Beer, T. and Cole, A.J. (1999) Pre- and postsynaptic activation of p44/42 MAP kinase in hippocampus after kainate-induced seizures. *Epilepsia*, 40: 21.

Parent, J.M. and Lowenstein, D.H. (1994) Treatment of refrac-

tory generalized status epilepticus with continuous infusion of midazolam. *Neurology*, 44(10): 1837–1840.

Patrylo, P.R., Schweitzer, J.S. and Dudek, F.E. (1999) Abnormal responses to perforant path stimulation in the dentate gyrus of slices from rats with kainate-induced epilepsy and mossy fiber reorganization. *Epilepsy Res.*, 36(1): 31–42.

Penner, M.R., Pinaud, R. and Robertson, H.A. (2001) Rapid kindling of the hippocampus protects against neural damage resulting from status epilepticus. *NeuroReport*, 12(3): 453–457.

Saffen, D.W., Cole, A.J., Worley, P.F., Christy, B.A., Ryder, K. and Baraban, J.M. (1988) Convulsant-induced increase in transcription factor messenger RNAs in rat brain. *Proc. Natl. Acad. Sci. USA*, 85: 7795–7799.

Sandberg, R., Yasuda, R., Pankratz, D.G., Carter, T.A., Del Rio, J.A., Wodicka, L., Mayford, M., Lockhart, D.J. and Barlow, C. (2000) Regional and strain-specific gene expression mapping in the adult mouse brain. *Proc. Natl. Acad. Sci. USA*, 97(20): 11038–11043.

Sasahira, M., Lowry, T., Simon, R.P. and Greenberg, D.A. (1995) Epileptic tolerance: Prior seizures protect against seizure-induced neuronal injury. *Neurosci. Lett.*, 185: 95–98.

Schafe, G.E., Atkins, C.M., Swank, M.W., Bauer, E.P., Sweatt, J.D. and LeDoux, J.E. (2000) Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *J. Neurosci.*, 20(21): 8177–8187.

Schauwecker, P.E. (2000) Seizure-induced neuronal death is associated with induction of c-Jun N-terminal kinase and is dependent on genetic background. *Brain Res.*, 884(1–2): 116–128.

Schauwecker, P.E. and Steward, O. (1997) Genetic determinants of susceptibility to excitotoxic cell death: implications for gene targeting approaches. *Proc. Natl. Acad. Sci. USA*, 94(8): 4103–4108.

Schmid, R., Tandon, P., Stafstrom, C.E. and Holmes, G.L. (1999) Effects of neonatal seizures on subsequent seizure-induced brain injury. *Neurology*, 53(8): 1754–1761.

Schwob, J.E., Fuller, T., Price, J.L. and Olney, J.W. (1980) Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: a histological study. *Neuroscience*, 5: 991–1014.

Selcher, J.C., Atkins, C.M., Trzaskos, J.M., Paylor, R. and Sweatt, J.D. (1999) A necessity for MAP kinase activation in mammalian spatial learning. *Learn. Memory*, 6(5): 478–490.

Sheline, Y.I., Sanghavi, M., Mintun, M.A. and Gado, M.H. (1999) Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J. Neurosci.*, 19(12): 5034–5043.

Sloviter, R.S. (1994) On the relationship between neuropathology and pathophysiology in the epileptic hippocampus of humans and experimental animals [Review]. *Hippocampus*, 4(3): 250–253.

Sutula, T.P., He, X.X., Cavazos, J. and Scott, G. (1988) Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science*, 239(4844): 1147–1150.

Vezzani, A., Sperk, G. and Colmers, W.F. (1999) Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends Neurosci.*, 22(1): 25–30.

Wasterlain, C.G. and Plum, F. (1973) Vulnerability of developing rat brain to electroconvulsive seizures. *Arch. Neurol.*, 29(1): 38–45.

Weiss, S., Cataltepe, O. and Cole, A.J. (1995) NGF reduced DNA fragmentation in CA1 neurons but not elsewhere after systemic kainate-induced seizures in the rat. *Soc. Neurosci. Abstr.*, 21: 985.

Willmore, L.J., Ballinger Jr., W.E., Boggs, W., Sypert, G.W. and Rubin, J.J. (1980) Dendritic alterations in rat isocortex within an iron-induced chronic epileptic focus. *Neurosurgery*, 7(2): 142–146.

Wuarin, J.-P. and Dudek, F.E. (1996) Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J. Neurosci.*, 16(14): 4438–4448.

QUERIES:

?#1: Should there be a ref. for Gale et al.? (page 19)
?#2: Should there be refs. for Dingledine and Lowenstein et al.? (page 21)