



## ‘NON-SYNAPTIC’ MECHANISMS IN SEIZURES AND EPILEPTOGENESIS\*

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The role of ‘non-synaptic’ mechanisms (i.e. those mechanisms that are independent of active chemical synapses) in the synchronization of neuronal activity during seizures and their possible contribution to chronic epileptogenesis are summarized. These ‘non-synaptic’ mechanisms include electrotonic coupling through gap junctions, electrical field effects (i.e. ephaptic transmission), and ionic interactions (e.g. increases in the extracellular concentration of  $K^+$ ). Several lines of evidence indicate that granule cells and pyramidal cells of the hippocampus, and probably other cortical neurons, can generate synchronized electrical activity after active chemical synaptic transmission has been blocked. This synchronized activity is sensitive to alterations in the size of the extracellular space, thus suggesting that electrical field effects and ionic mechanisms contribute to this synchronized activity. Recent studies also indicate that ‘non-synaptic’ synchronization is quite prominent early in development. Electrophysiological data from hippocampal and neocortical slices have led to a re-interpretation of the fast prepotentials (i.e. partial spikes) recorded in cortical pyramidal cells, suggesting that they may not be due to dendritic spike generation. Improvement in freeze-fracture ultrastructural techniques have led to a re-assessment of previous data on gap junctions in the nervous system and opened new approaches to the quantitative analysis and characterization of gap junctions on glia and neurons. Finally, new methods of dye/tracer coupling have the potential to provide a more rigorous basis for evaluating gap junctions and electrotonic communication between neurons in the mammalian central nervous system. Therefore, recent data continue to suggest that gap junctions and electrotonic coupling play an important role in neural integration, although additional studies using new techniques will be needed to address some of the controversial issues that have arisen over the last several decades. © 1998 Academic Press

KEYWORDS: gap junction; electronic coupling; ephaptic; field-effect; synchronization; epilepsy

### INTRODUCTION

#### *Background from the 1980s*

One of the oldest and most controversial issues in neuroscience has centered around the role of chemical versus electrical communication between neurons (the history has been reviewed elsewhere; e.g. Bennett, 1972, 1977, 1997; Dudek *et al.*, 1983, 1986). Today, neuroscientists generally accept that chemical synaptic transmission is the main form of communication under normal conditions, and that

an abnormality in chemical transmission is the basis of many neurological disorders. There can be no doubt that chemical synaptic transmission is the dominant mechanism of neuronal communication under most normal conditions. Our specific purpose here is to review briefly the long-standing and extensive body of data that supports the hypothesis that other ‘non-synaptic’ mechanisms contribute to the generation and spread of seizure activity (at least under certain conditions), and that electrotonic coupling through gap junctions may be an important form of electrical communication that can contribute to neuronal synchronization during epileptiform events (see also Carlen *et al.*, 1996 for recent review).

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### *Chemical synapses versus other forms of neuronal communication*

A wide variety of neurotransmitters and neuro-modulators mediate excitatory and inhibitory synaptic interactions among neurons, and all neurons receive chemical synaptic input from several types of fast and slow neurotransmitters and neuro-modulators. On the other hand, gap junctions between adult mammalian neurons are generally considered to be rare or essentially non-existent. Most authors do not consider gap junctions to be electrical synapses, nor do they consider electrotonic coupling potentials to be synaptic events. Others have challenged this view (e.g. Bennett, 1997). The fact that a gap junction between two neurons is an ultrastructural specialization that mediates electrical communication is an important argument for the concept of the 'electrotonic synapse.' Not only does an action potential in one member of a coupled pair cause an electrical event reminiscent of a 'synaptic potential' in the other member of the coupled pair, but electrotonic synapses have the potential to show remarkable forms of use-dependent plasticity (e.g. Bennett, 1972, 1977; Dudek *et al.*, 1988). The purpose of this article is not to debate the issue of whether gap junctions represent electrotonic synapses in a manner analogous to traditional chemical synapses, but rather to argue that electrotonic junctional communication in the mammalian brain may be one form of 'non-synaptic' transmission (i.e. independent of traditional chemical synaptic transmission) that contributes to the synchronization of epileptiform activity. Our use of the term 'non-synaptic' is not meant to exclude the gap junction and associated electrotonic coupling as a type of 'synapse' (i.e. we also view gap junctions as *electrical synapses*, in addition to critical components of 'mixed synapses'). From an experimental perspective, however, electrotonic coupling persists after treatments that block action potential-mediated chemical synaptic transmission (see below), and this has been an extremely useful approach for isolating 'non-synaptic' mechanisms of synchronization from chemical synaptic mechanisms.

Another mechanism of 'non-synaptic' communication between neurons is ephaptic transmission (i.e. electrical field effects). Although different authors may use the terms ephaptic transmission and field effects in different ways, their most general definition includes neuronal interactions mediated by electrical current flow through the extracellular space (see Jefferys, 1995 for review).

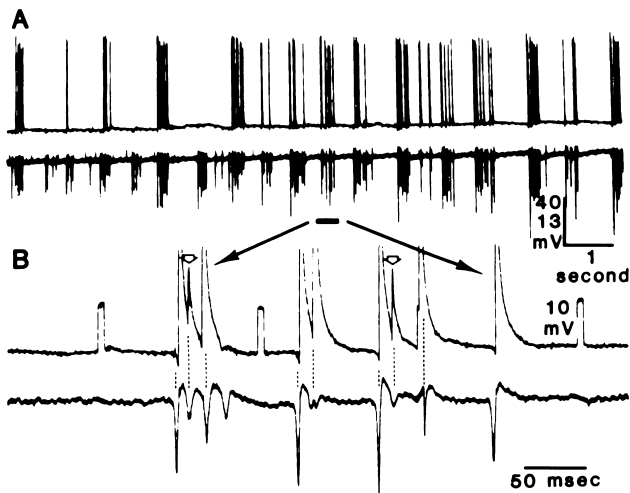
'Non-synaptic' communication also includes changes in the concentration of extracellular ions, most notably potassium ( $[K^+]_o$ ), which is likely to be important after high levels of neuronal activity. In this article, we will describe forms of epileptiform activity that have been shown to be independent of active chemical synaptic transmission, and then discuss the possible role of 'non-synaptic' mechanisms in synchronization of epileptiform activity, focusing at the end on gap junctions.

### *Role of chemical synapses and electrical interactions in epileptiform synchronization*

A large body of data generated in the 1970s and early 1980s, primarily based on *in vitro* experiments with hippocampal slices, provided strong evidence for the important role of recurrent excitatory synaptic mechanisms in the generation and synchronization of neuronal bursts during brief seizure-like events (e.g. Johnston and Brown, 1981; Traub and Wong, 1982; Miles and Wong, 1983, 1987; Christian and Dudek, 1988). In the early 1980s, however, at least three laboratories independently and almost simultaneously observed that blockade of active chemical synaptic transmission with low-calcium solutions (i.e. low  $[Ca^{2+}]_o$ ) led to prolonged bursts of large population spikes in CA1 pyramidal cells (Fig. 1A; Taylor and Dudek, 1982b, 1984a,b; Jefferys and Haas, 1982; Haas and Jefferys, 1984; Konnerth *et al.*, 1984, 1986; Yaari *et al.*, 1986; see also, Jensen and Yaari, 1988, 1997 for more recent studies). These experiments collectively provided unequivocal evidence that active chemical synaptic transmission is not necessary for synchronization of neuronal activity in the CA1 area of the hippocampus. What was unclear (and is still unresolved) is the role of the different electrical and ionic mechanisms in this synchronization process. A series of electrophysiological observations over the last 15 years has suggested that both ephaptic (i.e. electrical field effects) and ionic mechanisms contribute substantially to the synchronization mechanism; what remains unknown and controversial is the degree that gap junctions play a role in the synchronization of these events (see Traub *et al.*, 1985a,b).

### *Evidence for electrical interactions in cortical neurons*

The observation in the early 1980s of large spontaneous population spikes in extracellular recordings from the pyramidal cell layer of the CA1 area, when  $[Ca^{2+}]_o$  was reduced to block chemical



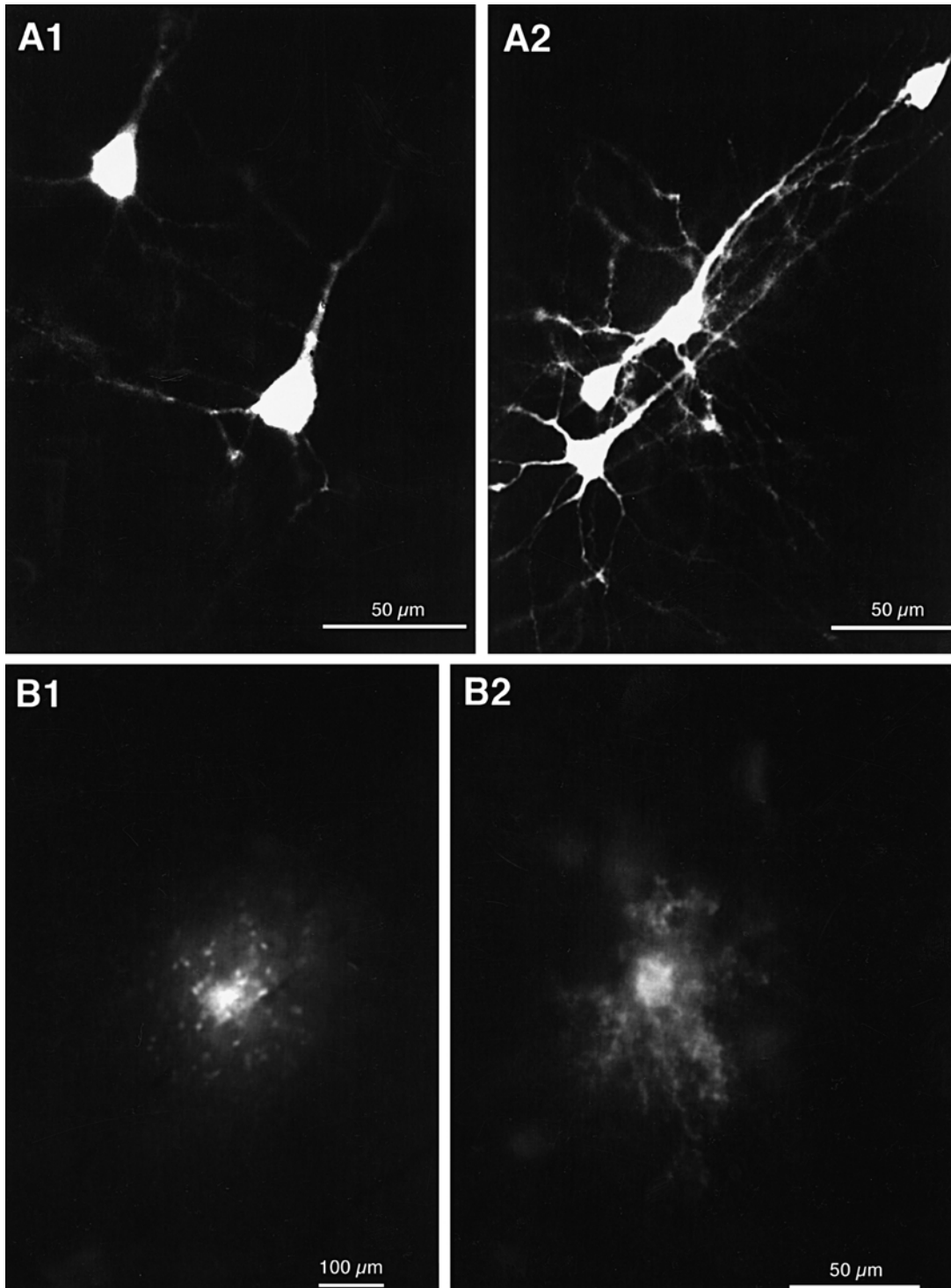
**Fig. 1.** Paired intracellular and extracellular recordings of spontaneously occurring bursts of synchronous action potentials in a hippocampal slice when chemical synaptic transmission was blocked with a low- $[Ca^{2+}]_o$  solution (i.e. 0.5 mM) containing  $Mg^{2+}$  (2.3 mM). A: bursts of intracellular action potentials (upper trace) were synchronous with most of the bursts of population spikes (lower trace). B: when the time scale was expanded (region indicated by bar in A), the intracellular action potentials and population spikes were synchronous (vertical dashed line). Subthreshold depolarizations (open arrows), which resembled fast prepotentials, were also synchronous with population spikes. Reprinted with permission from Taylor CP and Dudek FE, 1982. Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. *Science* **218**: 810–812. Copyright 1982 American Association for the Advancement of Science.

synaptic transmission, strongly suggested that some form of direct electrical communication is essential for fast synchronization of action potentials in pyramidal cells (Fig. 1). Low  $[Ca^{2+}]_o$  has long been known to increase membrane excitability (Frankenhaeuser and Hodgkin, 1957), and electrical interactions were hypothesized to synchronize the spontaneous action potentials. Electrophysiological experiments using differential recording (i.e. intracellular recording minus extracellular recording) indicated that the electrical fields associated with synchronized action potentials create a field-effect depolarization in inactive pyramidal cells, and thus serve as a mechanism for synchronizing the activity of neurons (e.g. Taylor and Dudek, 1982b, 1984a,b). Electrical field effects alone could conceivably account for the synchronization of action potentials, since they act instantaneously, and it has generally been considered that changes in  $[K^+]_o$  would likely be too slow to synchronize individual action potentials into a population spike.

Since earlier experiments in the hippocampus had revealed dye coupling after Lucifer Yellow

injections, as well as electrophysiological evidence for electrotonic coupling among pyramidal cells and granule cells (MacVicar and Dudek, 1980, 1982; Taylor and Dudek, 1982a), there existed the possibility that electrotonic coupling through gap junctions could synchronize neuronal activity and at least contribute to the 'non-synaptic' synchronization observed in low- $[Ca^{2+}]_o$  solutions. Intracellular recordings during synchronized activity in low- $[Ca^{2+}]_o$  solutions revealed that CA1 pyramidal cells generate fast prepotentials or partial spikes (Fig. 1B), as would be expected from electrotonic coupling. On the other hand, the dye coupling and electrophysiological evidence suggested that if electrotonic coupling were present in the hippocampus among pyramidal cells and granule cells, each neuron was coupled to at most only a few nearby neurons, and often to only one other neuron (similar to neocortex; see below and Fig. 2A). These data therefore implied that electrotonic coupling, on its own, was unlikely to explain the fast synchronization. These experiments, however, left open the possibility that electrotonic coupling through gap junctions among CA1 pyramidal cells could still contribute significantly to the synchronization. Although electrical mechanisms of neuronal communication were almost certainly responsible for the fast synchronization of action potentials needed to generate large population spikes in low- $[Ca^{2+}]_o$  solutions, what was unclear was whether gap junctions between CA1 pyramidal cells played an important role in this synchronization.

Similar evidence suggesting the presence of dye coupling and electrotonic coupling was generated nearly simultaneously in adult neocortex. Early ultrastructural data from primate neocortex had provided evidence for gap junctions between dendrites and somata (Sloper, 1972; Sloper and Powell, 1978). In neocortical slices from guinea pigs, Gutnick and Prince (1981) used electrical stimulation of nearby white matter to evoke antidromic short-latency depolarizations, which had the properties of electrotonic coupling potentials. They also found that injections of Lucifer Yellow into neocortical neurons often stained several neurons (i.e. dye coupling), and these coupled neurons tended to be oriented in a columnar manner (Fig. 2A). Similar to hippocampus, only a few dye-coupled neurons were found in adult tissue after intraneuronal injection of Lucifer Yellow. In glia, however, comparable injections revealed hundreds of stained astrocyte nuclei (Fig. 2B; Gutnick *et al.*, 1981); the widespread nature of the glial staining after intracellular injection of Lucifer Yellow into glia (see also, Binmoller and Muller,



**Fig. 2.** Dye coupling in neocortex. A: pyramidal cells. Intracellular injection of Lucifer Yellow into neocortical pyramidal cells often led to the staining of more than one neuron (A1), and the pattern of coupled neurons was often oriented in a columnar fashion (A2). From Gutnick and Prince, 1981. Reprinted with permission from Gutnick MJ and Prince DA, 1981. Dye-coupling and possible electrotonic coupling in the guinea pig neocortical slice. *Science* **211**: 67–70. Copyright 1981 American Association for the Advancement of Science. B: glial cells. When similar injections were undertaken in putative glial cells (i.e., astrocytes) of neocortical slices, many glial nuclei were observed (B1), thus supporting the hypothesis that the astrocytes (B2) are extensively coupled. Reprinted with permission from Gutnick MJ, Connors BW and Ranson BR, 1981. Dye-coupling between glial cells in the guinea pig neocortical slice. *Brain Research* **213**: 486–492, Copyright 1981 with permission from Elsevier Science.

1992) is consistent with the enormous number of gap junctions among glia.

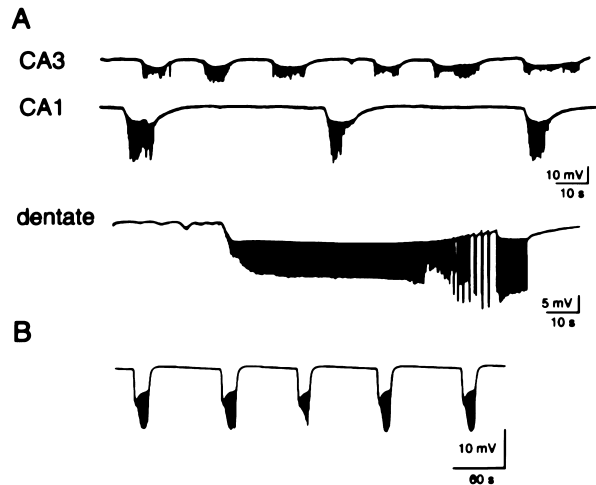
## RESULTS

### Data from the 1990s

Data generated in the 1980s set the stage for new findings in the 1990s further suggesting that 'non-synaptic' mechanisms of synchronization are important during epileptiform activity; these results are summarized briefly in this paragraph before describing the data in more detail later in this article. One line of recent investigation has been aimed at the issue of whether 'non-synaptic' mechanisms of synchronization are present throughout all the subfields of the hippocampus and in other cortical areas. Another set of experiments suggested that the high susceptibility to this form of 'non-synaptic' synchronization was due, at least in part, to the relatively small extracellular space between CA1 pyramidal cells (and possibly other hippocampal neurons). In other experiments, alterations in the osmolality of the medium, which would be expected to cause cell swelling or shrinkage, induced or blocked epileptiform activity, respectively. These data suggested, but did not prove, that ephaptic and ionic mechanisms play an important role in 'non-synaptic' synchronization of epileptiform activity in the hippocampus. Finally, a separate line of investigation revealed that the CA1 area and the dentate gyrus were particularly prone to 'nonsynaptic' synchronization early in development (i.e. during the first and second postnatal week). Since other studies indicated that electrotonic coupling is robust early in development, these data suggested a role for electrotonic coupling through gap junctions in the synchronization of the epileptiform events in low- $[Ca^{2+}]_o$  media, at least early in development. In spite of this possible temporal association, however, the role of gap junctions and electrotonic coupling in synchronization, even early in development, remains unclear.

### 'Nonsynaptic' synchronization beyond the CA1 area

**Dentate gyrus and the CA3 area.** Although a brief report in the 1980s provided evidence that the CA3 area and the dentate gyrus could show synchronized electrical activity in low- $[Ca^{2+}]_o$  solutions that blocked active chemical synaptic transmission (Snow and Dudek, 1984), these earlier studies were



**Fig. 3.** Spontaneously occurring bursts without active chemical synaptic transmission in neurons throughout the hippocampus. A: a combination of low  $[Ca^{2+}]_o$  and high  $[K^+]_o$  caused bursts in CA3, CA1, and the dentate gyrus. With 0-added  $Ca^{2+}$  and 5 mM  $[K^+]_o$ , CA3 and CA1 displayed prolonged spontaneous bursts which were not present in the dentate gyrus (not shown). With 9 mM  $[K^+]_o$  and 0-added  $Ca^{2+}$ , prolonged spontaneous field-potential bursts were present in the dentate gyrus, but did not occur in the CA3 or CA1 areas (not shown). Vertical calibration bar is 5 mV for the dentate gyrus, and 10 mV for CA3 and CA1. Reprinted with permission from Schweitzer JS, Patrylo PR, Dudek FE, 1992. Prolonged field bursts in the dentate gyrus: Dependence on low calcium, high potassium, and nonsynaptic mechanisms. *J. Neuroscience* 68: 2016. Copyright 1992 American Physiological Society. B: prolonged bursts of population spikes could occur in normal  $[Ca^{2+}]_o$  (i.e. 1.3 mM) and high  $[K^+]_o$  (i.e. 12 mM), with 30  $\mu$ M bicuculline, 50  $\mu$ M AP5 and 50  $\mu$ M DNQX to block amino acid-mediated fast synaptic transmission postsynaptically. Reprinted with permission from Patrylo PR, Schweitzer JS, Dudek FE, 1994. Potassium-dependent prolonged field bursts in the dentate gyrus: Effects of extracellular calcium and amino acid receptor antagonists. *Neuroscience* 61: 13–19. Copyright 1994 with permission from Elsevier Science.

not able to show consistently robust synchronized activity similar to what had been seen in the CA1 area. Additional studies in which  $[Ca^{2+}]_o$  was systematically altered revealed that under certain conditions, the dentate gyrus and the CA3 area could show profound synchronized electrical activity when chemical synaptic transmission was blocked (Fig. 3; Schweitzer *et al.*, 1992). In this study, and an earlier one on CA1 (Dudek *et al.*, 1990), fast glutamatergic and/or GABAergic transmission was also blocked postsynaptically with amino acid receptor antagonists. Those studies showed that all of the subfields of the hippocampus were capable of generating robust synchronized activity without active chemical synapses, so long as  $[Ca^{2+}]_o$  and  $[K^+]_o$  were adjusted appropriately. When  $[Ca^{2+}]_o$  was nominally reduced to zero and  $[K^+]_o$  was about 5 mM, the CA3 area but not the dentate

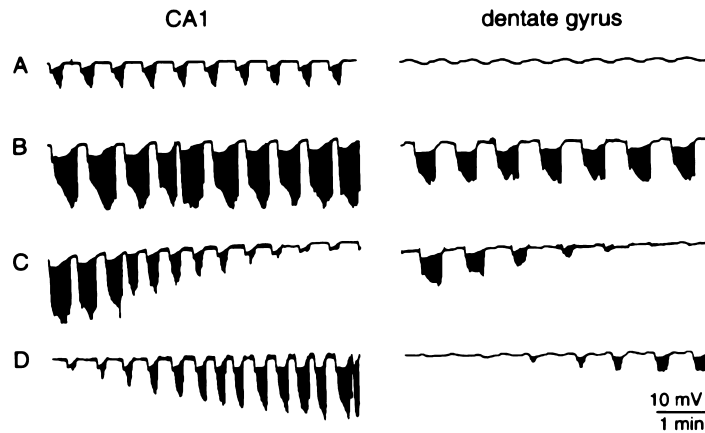
gyrus showed robust, spontaneous, seizure-like activity. When  $[K^+]_o$  was raised to 9 mM, however, the dentate gyrus but not the CA3 area showed prolonged epileptiform bursting, which suggested that this difference in susceptibility to 'non-synaptic' seizure generation was related at least in part to the differences in resting potential of these two types of neurons. Thus, it appeared that 'non-synaptic' mechanisms of synchronization were present throughout the hippocampus (see also, Richardson and O'Reilly, 1995; Pan and Stringer, 1996). Subsequent studies showed that these mechanisms of 'non-synaptic' synchronization were present when  $[K^+]_o$  and  $[Ca^{2+}]_o$  were within the range known to occur during seizure activity and chemical synaptic transmission was blocked postsynaptically with amino acid receptor antagonists (Patrylo *et al.*, 1994). This observation supports the hypothesis that these 'nonsynaptic' mechanisms are likely to be operative during seizure activity, and that they are not just a secondary effect of greatly altered  $[Ca^{2+}]_o$  and  $[K^+]_o$ .

*Medial entorhinal cortex.* The experiments in hippocampal slices suggested that other cortical areas might also show synchronized activity when active chemical synapses were blocked with low- $[Ca^{2+}]_o$  solutions. Therefore, similar experiments were conducted in the medial entorhinal cortex, an area of temporal cortex near the dentate gyrus. The medial entorhinal cortex is intermediate between the tight packing of pyramidal cell bodies in the hippocampus and the more widely dispersed distribution of pyramidal cells characteristic of neocortex. In these experiments, field-potential and multiple-unit recordings showed that synchronization could also occur in the medial entorhinal cortex (Patrylo *et al.*, 1996). Even though the field potentials were substantially smaller than those in the hippocampus, bursts of population spikes were still clearly observed in slices from adult rats. Furthermore, the high-gain multiple-unit recordings showed that occasionally synchronization occurred only among small groups of neurons, when field potentials were small or nonexistent (in both the CA1 area and the medial entorhinal cortex). Because synchronized activity can occur in an area where electrical field effects are expected to be small, other 'non-synaptic' mechanisms are likely involved. One possibility is that electrotonic coupling of small clusters of neurons in medial entorhinal cortex can generate small domains of synchronously active neurons. It is not known, however, if other areas of adult cortex with more traditional cortical layering are capable of generat-

ing synchronized electrical activity when chemical synaptic transmission is blocked.

### *Role of extracellular space*

Based primarily on early anatomical studies (Green, 1964; Green and Maxwell, 1961), it has been known for decades that the somata of hippocampal pyramidal cells and dentate granule cells show extraordinarily tight packing. Green (1960, 1964) actually proposed that the tight packing of cell bodies in the hippocampus could lead to electrical interactions, which could potentially contribute to synchronization of electrical activity. This feature, in addition to the parallel arrangement of the neurons, is considered to be the basis for the extraordinarily large field potentials characteristic of the hippocampus. Early studies aimed at measuring changes of the extracellular space in hippocampus and neocortex provided physiological evidence that the extracellular space in the hippocampus is particularly small (Dietzel *et al.*, 1980, 1989; Lux *et al.*, 1986). McBain and co-workers (1990) showed that the extracellular volume fraction was approximately 0.12 in the CA1 area compared to 0.18 or larger in other areas of the brain. Traynelis and Dingledine (1988) showed that extracellular resistance increased at the beginning of high  $[K^+]_o$ -induced seizure activity in the CA1 area. They also showed that bathing solutions made hyperosmolar with impermeant solutes block high- $[K^+]_o$ -induced seizure activity in CA1 (Traynelis and Dingledine, 1989). Studies with low- $[Ca^{2+}]_o$  solutions have shown that these  $K^+$ -induced synchronous bursts are also blocked with hyperosmolar solutions that cause cell shrinkage, and furthermore, that dilute media (which cause cell swelling) can induce seizure activity (Fig. 4; Dudek *et al.*, 1990; Roper *et al.*, 1992). More recently, treatment with furosemide, which increases extracellular space, blocks synchronization independent of alterations in excitability (Hochman *et al.*, 1996). Therefore, anatomical and physiological evidence indicates that: (1) the extracellular space is small in the hippocampus; (2) intense electrical activity causes cell swelling and a decrease in the extracellular space; and (3) experimental manipulations that cause cell shrinkage and increased extracellular space can block seizure activity, independent of chemical synapses (see also, Andrew *et al.*, 1989). Since a decrease in extracellular space should enhance ionic and ephaptic effects, these forms of neuronal communication probably play important roles in the



**Fig. 4.** Changes in the osmolality of the extracellular medium altered seizure-like activity in CA1 and the dentate gyrus. A: in low- $[Ca^{2+}]_o$  solution, spontaneously occurring bursts were present in CA1 but not the dentate gyrus. B: with dilution of the extracellular medium from 308 mOsm to 254 mOsm, the CA1 bursts increased in amplitude and duration, and spontaneously occurring bursts were present in the dentate gyrus. C: in both CA1 and the dentate gyrus, bursts were abolished when mannitol (20 mM) was added to increase extracellular osmolality. D: dilution of the extracellular fluid again caused bursts. Reprinted with permission from Roper SN, Obenaus A and Dudek FE, 1992. Osmolality and nonsynaptic epileptiform bursts in rat CA1 and dentate gyrus. *Ann Neurol* 31: 81–85. Copyright 1992 Lippincott Williams and Wilkins.

generation and synchronization of epileptiform activity, independent of chemical synapses.

#### Development

One important issue in epilepsy research is why the immature nervous system is more susceptible to seizure generation. It is well known that groups of cells tend to be coupled early in development, and several experiments have provided evidence that cortical neurons are coupled in the first postnatal weeks in the rat, and that this coupling declines with age (e.g. Connors *et al.*, 1983; Yuste *et al.*, 1995; Rorig and Sutor, 1996). Accordingly, one issue is whether the increased seizure susceptibility of the immature hippocampus is still present when chemical synaptic mechanisms have been blocked pharmacologically. Albrecht and Heinemann (1989) provided evidence that epileptiform activity occurs at higher concentrations of  $[Ca^{2+}]_o$  in the CA1 region of hippocampal slices from rats at 7–8 and 15–16 days of age than in hippocampal slices from adults. They also described unusually prolonged epileptiform discharges (i.e., 10 min) in the CA1 area from rats that were 8–9 days old. Roper *et al.* (1993) found a developmental window at 2–3 weeks in the rat where both the CA1 area of the hippocampus and the dentate gyrus are more susceptible to epileptiform bursting in low- $[Ca^{2+}]_o$  solutions. In CA1, the epileptiform activity was also more robust in the immature animals. These

findings indicate that the increased seizure susceptibility of the immature brain is present even when chemical synaptic transmission is blocked. Although an increase in electrotonic coupling through gap junctions among hippocampal neurons is one of many possible mechanisms that could contribute to this lower seizure threshold, further studies are needed to evaluate this hypothesis.

## DISCUSSION

### *Ongoing issues, and research for 2000 and beyond*

*The fast prepotential: dendritic spike or electrotonic coupling?* In the 1960s, Kandel and Spencer published a series of papers describing the electrophysiological properties of hippocampal pyramidal cells studied in the cat *in vivo*. They proposed that action potentials could be initiated by two types of events—fast and slow prepotentials. They concluded that slow prepotentials were EPSPs and the fast prepotentials were due to the passive propagation of action potentials initiated in the proximal apical dendrite (Spencer and Kandel, 1961). Several studies later reported spike-like events in the dendrites of hippocampal pyramidal cells (Wong *et al.*, 1979) and dentate granule cells (Fricke and Prince, 1984). However, it was impossible with recordings from single intracellular electrodes to determine conclusively whether these events were generated in the dendrite or the soma.

Some of these events were relatively small, or at least were not full-amplitude action potentials, which is consistent with the hypothesis that the events were generated in the soma and passively spread back into the dendrites, rather than actively initiated in the dendrite and propagated to the soma. Experiments by [Stuart and Sakmann \(1994\)](#) have directly reexamined the issue of dendritic spike generation in cortical neurons, and provided direct evidence that sodium-mediated action potentials in neocortical slices are normally elicited in the axosomatic region and back-propagated into the dendrites. They found that even when depolarizing current was injected directly into the dendrite, the sodium spike was initiated near the soma, and later propagated out the dendrite. Subsequent studies ([Stuart et al., 1997a,b](#)) in older animals and at more physiological temperatures have shown that the distal dendrite can initiate sodium-mediated action potentials, but the somatic response of neocortical pyramidal cells during these dendritic spikes does not resemble a fast prepotential. Although some fast prepotentials may ultimately be due to sodium-mediated action potentials generated in dendrites, these recent data require reexamination of data from older experiments with sharp-electrode intracellular recordings from dendrites.

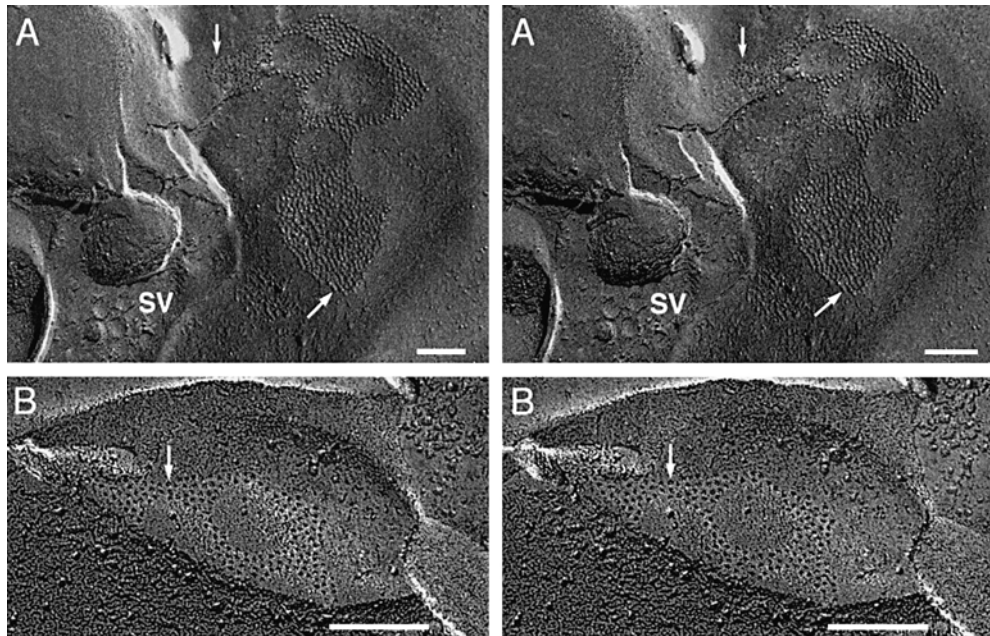
The initial indirect evidence that fast prepotentials were dendritic spikes was that they were evoked by orthodromic but not by antidromic stimulation ([Spencer and Kandel, 1961](#)). Subsequently, however, [Schwartzkroin and Prince \(1980\)](#) noted that these events could sometimes be evoked with antidromic stimulation in CA1 pyramidal cells recorded in hippocampal slices. This observation is difficult to reconcile with dendritic generation of sodium spikes. It is, however, consistent with electrotonic coupling, as is the observation that these events can sometimes be recorded at 'anodal break' (i.e. the end of a hyperpolarizing pulse). Several studies have now observed fast prepotentials or partial spikes in hippocampal pyramidal cells and neocortical neurons in response to antidromic stimulation, and these spike-like events are sometimes resistant to prior current-evoked action potentials, thus suggesting that they are due to electrotonic coupling (i.e. the indirect test; [Taylor and Dudek, 1982a](#); [Gutnick and Prince, 1981](#)). Dual intracellular recordings also suggested that hippocampal pyramidal cells are electronically coupled ([MacVicar and Dudek, 1981](#)), but these studies have been criticized because the possibility that the electrodes were actually in only one neuron was not unequivocally ruled out. More recently

current-evoked depolarization in CA1 pyramidal cells caused burst discharges with fast prepotentials, which has been correlated with Lucifer Yellow dye coupling ([Baimbridge et al., 1991](#)). [Carlen and coworkers \(Perez-Velazquez et al., 1994; Valiante et al., 1995; see also, Carlen et al., 1996; Vigmond et al., 1997\)](#) have recently studied 'spikelets' and electrical coupling in hippocampal pyramidal cells with whole-cell patch-clamp techniques and computer simulations, and have provided new evidence that electrical interactions contribute to synchronization of epileptiform events. The controversies on the previous electrophysiological and dye coupling data are numerous, and additional work with more modern techniques is required to address this issue.

Recent studies with patch-clamp techniques and computer modeling have provided new evidence for electrotonic coupling among hippocampal pyramidal cells, and have suggested that gap junctions interconnect axons ([Draguhn et al., 1998](#); [Traub et al., 1999](#)).

*The need for ultrastructural data: grid-mapped freeze fracture and immunolabeling?* The studies from the 1980s attempted to use several lines of evidence to determine whether cortical neurons were dye coupled and electrotonically coupled through gap junctions. Freeze-fracture images were considered to provide evidence for gap junctions on CA3 hippocampal pyramidal cells ([Schmalbruch and Jahnsen, 1981](#)) and dentate granule cells ([MacVicar and Dudek, 1982](#)). In these studies, gap junctions were found on large somata in or near the pyramidal and granule cell layers, respectively, and these cells appeared to be closely associated with chemical synapses of nerve terminals. Therefore, these data were interpreted as showing that gap junctions were on the somata of hippocampal pyramidal cells and dentate granule cells. Using thin-section electron microscopy, gap junctions have been found on interneurons in the hippocampus ([Kosaka, 1983a,b](#)), which is consistent with other more recent data that the interneurons are dye-coupled and electrotonically coupled ([Michelson and Wong, 1994](#); [Traub, 1995](#); [Strata et al., 1997](#)). Thus, ultrastructural evidence has been available to suggest the presence of gap junctions on hippocampal neurons.

Recent improvements in the freeze-fracture technique, particularly in the use of Lexan coating, have allowed preparation of large replicas. By mapping those grids using confocal microscopy, it is now possible to identify more completely the precise location of different cell types in terms of



**Fig. 5.** Stereoscopic images of freeze-fracture replicas of gap junctions between unidentified hippocampal neurons. A: P-face and E-face image of gap junction on 3- $\mu$ m diameter dendrite. SV=synaptic vesicles in adjacent chemical synapse. Arrows point to margins of gap junction.  $\times 65,000$ . B: E-face image of gap junction on small dendrite. Arrows point to 'pegs' in E-face pits.  $\times 130,000$ . Reprinted with permission from Rash JE, Duffy HS, Dudek FE, Bilhartz BL, Whalen LR, Yasumura T, 1997. Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a 'panglial syncytium' that is not coupled to neurons. *J Comp Neurol* **388**: 265–292. Copyright 1997 with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

the anatomical features of the hippocampus or other structures (Rash *et al.*, 1995, 1996, 1997). Furthermore, detailed criteria for identifying neurons versus different types of glia (Rash *et al.*, 1997) have led to a reexamination of earlier freeze fracture data, with the conclusion that gap junctions reported to be on hippocampal pyramidal cells (Schmalbruch and Jahnsen, 1981) and dentate granule cells (MacVicar and Dudek, 1982) were actually on oligodendrocytes (or were on cells that were not identifiable). New evidence for gap junctions on hippocampal neurons has been obtained with grid-mapped freeze fracture (Fig. 5; Rash *et al.*, 1997), but because the gap junctions were on dendrites (rather than somata), it is not clear whether these particular gap junctions were on principal cells (i.e. granule cells and pyramidal cells) or on interneurons. Recent studies with grid-mapped freeze fracture have shown that different connexins in glial gap junctions can be immunolabeled (Rash *et al.*, 1997); this technique should allow a simpler, yet more rigorous analysis of whether hippocampal pyramidal cells and dentate granule cells have gap junctions, and should lead to identification of the particular connexins involved in the putative neuronal gap junctions.

*The next wave of tracer-coupling and electrophysiological studies?* Studies using dye coupling with Lucifer Yellow or tracer coupling with biocytin or neurobiotin have been plagued with two fundamental problems: (1) because sharp electrodes or even patch pipettes have to be advanced through many tens of micrometers (even hundreds of micrometers) of tissue before obtaining a recording, it has been suspected that multiple staining of neurons could be due to an artifact from damage of neurons as an electrode is advanced through the tissue (e.g. see Alger *et al.*, 1983; Knowles *et al.*, 1982; Gutnick *et al.*, 1985; Schneider, 1992); and (2) even in well-controlled systems, such as cell cultures, it is known that some cell pairs that are electrotonically coupled are not necessarily dye coupled. Although there have been a myriad of discussions on the details of these two issues, ultimately many workers have considered this type of work to be equivocal, unless other supporting data have been available. The problem is that both electrophysiological and ultrastructural approaches have had potential criticisms also.

A variety of techniques are now available to address these issues. (1) Gap junctions are known to be sensitive to intracellular pH and other treatments. Accordingly, dye coupling of pyramidal

cells has been shown to be reduced by intracellular acidification (Gutnick and Lobel-Yaakov, 1983; MacVicar and Jahnsen, 1985) and enhanced by high-pH medium (Church and Baimbridge, 1991). (2) Gap junctions allow passage of small molecules (<1000 M<sub>r</sub>) but not larger ones. Therefore, Lo Turco and Kriegstein (1991) injected both Lucifer Yellow and horseradish peroxidase (HRP, a large protein expected not to cross gap junctions) into immature neocortical neurons; they showed that although Lucifer Yellow stained multiple neurons, HRP was only found in a single neuron that was presumably the one injected with the markers. (With this approach, adequate HRP staining along the dendrites and axons has to be present to insure that the observation of HRP in a single cell is not merely due to the much slower diffusion of the larger molecule.) (3) The potential for artifactual staining would be reduced if neurons were studied at the surface of the slice under direct visualization, and dye was prevented from leakage out of the pipette until the recording was actually achieved. Dean and colleagues (1997) have used direct visualization of the patch pipette with differential interference contrast optics during recording of visually identified neurons in thin slices of brain stem. This strategy minimized the potential of artifactually staining other neurons because the recording was obtained under direct visual observation near the surface of the slice. These workers also recorded from neurons using amphotericin perforated-patch pipettes that allowed them to first record from a neuron and identify whether it had partial spikes; they then ruptured the membrane, obtained a whole-cell recording, and stained the neuron with Lucifer Yellow and/or biocytin. They found that the neurons with fast prepotentials or partial spikes were almost always the neurons that showed dye/tracer coupling. These authors were also able to follow the movement of Lucifer Yellow into the coupled neurons under direct visual observation. Therefore, two particularly powerful approaches for dealing with potential artifacts in dye/tracer coupling studies are to use both a large molecule and a small molecule simultaneously, and to directly visualize the patch pipette and recorded neuron with thin slices using perforated-patch techniques.

These experiments provide the possibility of more rigorously testing the hypothesis of electrotonic coupling among mammalian brain neurons through the use of dual whole-cell patch-clamp recording in thin slices. Because one can directly visualize the recorded neurons, it should be possible in the future to record from pairs of electro-

tonically coupled cells, and use gap junction permeant and impermeant tracers to show that apparent electrotonic coupling is associated with tracer coupling for small molecules, but not large molecules (i.e., coupling is mediated by gap junctions and not an artifactual pathway from cell injury).

*Seizures versus epileptogenesis.* Seizures represent abnormal hypersynchronous and hyperactive electrical behavior of cortical neurons that can result from an acute insult or be caused by a variety of pharmacological treatments. Epilepsy, however, is a chronic condition with an increased susceptibility to seizures. Thus, the mechanisms operative in acute seizures induced experimentally in otherwise normal brain tissue are not necessarily indicative of the basis for chronic epileptogenesis. Although numerous studies have suggested a role for electrotonic coupling through gap junctions in epileptogenesis, one recent study has provided experimental evidence in support of this hypothesis. Colling *et al.* (1996) used the tetanus toxin model of chronic temporal lobe epilepsy and showed a significant increase in dye coupling of pyramidal cells in hippocampal slices. Further studies on models of chronic epilepsy are needed to determine if the increased seizure susceptibility is due to an increase in electrotonic coupling through gap junctions.

## CONCLUSIONS

The historical issue of whether neurons communicate by chemical versus electrical means has been resolved in that there is no question that chemical transmission is the primary method of communication between neurons in the mammalian nervous system. What continues to remain unsettled, however, is the degree to which electrical communication through gap junctions is important. Numerous studies in the hippocampus, neocortex, and elsewhere have provided evidence for dye/tracer coupling and electrotonic coupling through gap junctions. Nonetheless, this work is controversial, not only with regard to the relative importance of gap junctions among neurons, but even to the issue of whether gap junctions and electrotonic coupling are present in the adult mammalian nervous system. Numerous groups studying hippocampal slices have provided strong evidence that all of the subfields of the hippocampus, including the dentate gyrus and even the medial entorhinal cortex, can generate

synchronized electrical activity after chemical synaptic transmission has been blocked, both pre- and postsynaptically. One view is that this synchronization can be accounted for completely by ephaptic and ionic effects among hippocampal neurons without invoking a role for gap junctions between neurons. Other lines of evidence suggest that blocking gap junctional communication eliminates synchronized epileptiform activity, thus suggesting that gap junctions among hippocampal pyramidal cells are critical for the synchronization process. What is needed now is a detailed re-examination of this question using the new techniques in electrophysiology, tracer coupling, and ultrastructure.

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## REFERENCES

- ALBRECHT D, HEINEMANN U, 1989. Low calcium-induced epileptiform activity in hippocampal slices from infant rats. *Dev Brain Res* **48**: 316–320.
- ALGER BE, MCCARREN M, FISHER RS, 1983. On the possibility of simultaneously recording from two cells with a single microelectrode in the hippocampal slice. *Brain Res* **270**: 137–141.
- ANDREW RD, FAGAN M, BALLY BA, ROSEN A, 1989. Seizure susceptibility and the osmotic state. *Brain Res* **498**: 175–180.
- BAIMBRIDGE KG, PEET MJ, MCLENNAN H, CHURCH J, 1991. Bursting response to current-evoked depolarization in rat CA1 pyramidal neurons is correlated with Lucifer Yellow dye coupling but not with the presence of Calbindin-D28. *Synapse* **7**: 269–277.
- BENNETT MVL, 1972. A comparison of electrically and chemically mediated transmission. In: Pappas GD, Purpura DP, eds. *Structure and Function of Synapses*. New York, Raven Press. 221–256.
- BENNETT MVL, 1977. Electrical transmission: a functional analysis and comparison with chemical transmission. In: Kandel ER, ed. *Handbook of Physiology, Section I: The Nervous System, Vol. 1, Cellular Biology of Neurons, Part 1*. Bethesda, American Physiological Society. 357–416.
- BENNETT MVL, 1997. Gap junctions as electrical synapses. *J Neurocytol* **26**: 349–366.
- BINMOLLER FJ, MULLER CM, 1992. Postnatal development of dye-coupling among astrocytes in rat visual cortex. *Glia* **6**: 127–137.
- CARLEN PL, PEREZ-VALAZQUEZ, VALIANTE TA, JAHROMI SS, BARDAKJIAN BK, 1996. Electric coupling in epileptogenesis. In: Spray DC, Dermietzel R, eds. *Gap Junctions in the Nervous System*. R.G. Landes Company. 289–299.
- CHRISTIAN EP, DUDEK FE, 1988. Characteristics of local excitatory circuits studied with glutamate microapplication in the CA3 area of rat hippocampal slices. *J Neurophysiol* **59**: 90–109.
- CHURCH J, BAIMBRIDGE G, 1991. Exposure to high-pH medium increases the incidence and extent of dye coupling between rat hippocampal CA1 neurons in vitro. *J Neurosci* **11**: 3289–3295.
- COLLING SB, MAN WD-C, DRAGUHN A, JEFFERYS JGR, 1996. Dendritic shrinkage and dye-coupling between rat hippocampal CA1 pyramidal cells in the tetanus toxin model of epilepsy. *Brain Res* **741**: 38–43.
- CONNORS BW, BERNARDO LS, PRINCE DA, 1983. Coupling between neurons of the developing rat neocortex. *J Neurosci* **3**: 773–782.
- DEAN JB, HUANG R-Q, ERLICHMAN JS, SOUTHARD TL, HELLARD DT, 1997. Cell-cell coupling occurs in dorsal medullary neurons after minimizing anatomical-coupling artifacts. *Neuroscience* **80**: 21–40.
- DIETZEL I, HEINEMANN U, HOFMEIER G, LUX HD, 1980. Transient changes in the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. *Exp Brain Res* **40**: 432–439.
- DIETZEL I, HEINEMANN U, LUX HD, 1989. Relations between slow extracellular potential changes, glial potassium buffering, and electrolyte and cellular volume changes during neuronal hyperactivity in cat brain. *Glia* **2**: 25–44.
- DRAGHUN A, TRAUB RD, SCHMITZ D, JEFFERYS JGR, 1998. Electrical coupling underlies high frequency oscillations in the hippocampus in vitro. *Nature* **394**: 189–192.
- DUDEK FE, ANDREW RD, MACVICAR BA, SNOW RW, TAYLOR CP, 1983. Recent evidence for and possible significance of gap junctions and electrotonic synapses in the mammalian brain. In: Jasper HH, van Gelder NM, eds. *Basic Mechanisms of Neuronal Hyperexcitability*. In the series Chan-Palay V, Palay SL, eds. *Neurology and Neurobiology*. New York, Alan R. Liss. 31–73.
- DUDEK FE, GRIBKOFF VK, CHRISTIAN EP, 1988. Mechanisms of potentiation independent of chemical synapses. In: Landfield PW, Deadwyler SA, eds. *Long-Term Potentiation: From Biophysics to Behavior*. In the series Chan-Palay V, Palay SL, eds. *Neurology and Neurobiology*. New York, Alan R. Liss. 439–46.
- DUDEK FE, OBENAU A, TASKER JG, 1990. Osmolality-induced changes in extracellular volume alter epileptiform bursts independent of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. *Neurosci Lett* **120**: 267–270.
- DUDEK FE, SNOW RW, TAYLOR CP, 1986. Role of electrical interactions in synchronization of epileptiform bursts. In: Delgado-Escueta AV, Ward AA, Jr, Woodbury DM, Porter RJ, eds. *Basic Mechanisms of the Epilepsies: Molecular and Cellular Approaches*, in the series Advances in Neurology. New York, Raven Press. 593–617.
- FRANKENHAEUSER B, HODKIN AL, 1957. The action of calcium on the electrical properties of squid axons. *J Physiol* **137**: 218–244.
- FRICKE RA, PRINCE DA, 1984. Electrophysiology of dentate gyrus granule cells. *J Neurophysiol* **51**: 195–209.
- GREEN JD, 1960. The hippocampus. *Handbook of Physiology, Section I: Neurophysiology*, vol II, ed by Field J, pp 1373–1389. American Physiological Society, Washington DC.
- GREEN JD, 1964. The hippocampus. *Physiol Rev* **44**: 561–608.
- GREEN JD, MAXWELL DS, 1961. Hippocampal electrical activity I. Morphological aspects. *Electroenc Clin Neurophysiol* **13**: 868–879.

- GUTNICK MJ, CONNORS BW, RANSOM BR, 1981. Dye-coupling between glia cells in the guinea pig neocortical slice. *Brain Res* **213**: 486–492.
- GUTNICK MJ, LOBEL-YAAKOV R, 1983. Carbon dioxide uncouples dye-coupled neuronal aggregates in neocortical slices. *Neurosci Lett* **42**: 197–200.
- GUTNICK MJ, LOBEL-YAAKOV R, RIMON G, 1985. Incidence of neuronal dye-coupling in neocortical slices depends on the plane of section. *Neuroscience* **15**: 659–666.
- GUTNICK MJ, PRINCE DA, 1981. Dye coupling and possible electrotonic coupling in the guinea pig neocortical slice. *Science* **211**: 67–70.
- HAAS HL, JEFFERYS JGR, 1984. Low-calcium field burst discharges of CA1 pyramidal neurones in rat hippocampal slices. *J Physiol* **354**: 185–201.
- HOCHMAN DW, BARABAN SC, OWENS JWM, SCHWARTZKROIN PA, 1996. Dissociation of synchronization and excitability in furosemide blockade of epileptiform activity. *Science* **270**: 99–102.
- JEFFERYS JGR, 1995. Nonsynaptic modulation of neuronal activity in the brain: Electric currents and extracellular ions. *Physiol Rev* **75**: 689–723.
- JEFFERYS JGR, HAAS HL, 1982. Synchronized bursting of CA1 hippocampal pyramidal cells in the absence of synaptic transmission. *Nature* **300**: 448–450.
- JENSEN MS, YAARI Y, 1997. Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. *J Neurophysiol* **77**: 1224–1233.
- JENSEN MS, YAARI Y, 1988. The relationship between interictal and ictal paroxysms in an in vitro model of focal hippocampal epilepsy. *Ann Neurol* **24**: 591–598.
- JOHNSTON D, BROWN TH, 1981. Giant synaptic potential hypothesis for epileptiform activity. *Science* **211**: 294–297.
- KNOWLES WD, FUNCH PG, SCHWARTZKROIN PA, 1982. Electrotonic and dye coupling in the hippocampal slice. *Neuroscience* **7**: 1713–1722.
- KONNERTH A, HEINEMANN U, YAARI Y, 1984. Slow transmission of neural activity in hippocampal area CA1 in absence of active chemical synapses. *Nature* **307**: 69–71.
- KONNERTH K, HEINEMANN U, YAARI Y, 1986. Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. I. Development of seizure like activity in low extracellular calcium. *J Neurophysiol* **56**: 409–423.
- KOSAKA T, 1983a. Gap junctions between nonpyramidal cell dendrites in the rat hippocampus (CA1 and CA3 regions). *Brain Res* **271**: 157–161.
- KOSAKA T, 1983b. Neuronal gap junctions in the polymorph layer of the rat dentate gyrus. *Brain Res* **277**: 347–351.
- LO TURCO JJ, KRIEGSTEIN AR, 1991. Clusters of coupled neuroblasts in embryonic neocortex. *Science* **252**: 563–566.
- LUX HD, HEINEMANN U, DIETZEL I, 1986. Ionic changes and alterations in the size of the extracellular space during epileptic activity. In: Delgado-Escueta AV, Ward AA, Jr, Woodbury DM, Porter RJ, eds. *Advances in Neurology*. New York, Raven Press. 619–639.
- MACVICAR BA, DUDEK FE, 1980. Dye coupling between CA3 pyramidal cells in slices of rat hippocampus. *Brain Res* **196**: 494–497.
- MACVICAR BA, DUDEK FE, 1981. Electrotonic coupling between pyramidal cells: a direct demonstration in rat hippocampal slices. *Science* **213**: 782–785.
- MACVICAR BA, DUDEK FE, 1982. Electrotonic coupling between granule cells of rat dentate gyrus: physiological and anatomical evidence. *J Neurophysiol* **47**: 579–592.
- MACVICAR BA, JAHNSEN H, 1985. Uncoupling of CA3 pyramidal neurons by propionate. *Brain Res* **330**: 141–145.
- MCBAIN CJ, TRAYNELIS SF, DINGLELINE R, 1990. Regional variation of extracellular space in the hippocampus. *Science* **249**: 674–677.
- MICHELSON HB, WONG RKS, 1994. Synchronization of inhibitory neurons in the guinea pig hippocampus in vitro. *J Physiol* **477**: 35–45.
- MILES R, WONG RKS, 1983. Single neurones can initiate synchronized population discharge in the hippocampus. *Nature* **306**: 371–373.
- MILES R, WONG RKS, 1987. Inhibitory control of local excitatory circuits in the guinea pig hippocampus. *J Physiol* **388**: 611–629.
- PAN E, STRINGER JL, 1996. Burst characteristics of dentate gyrus granule cells: evidence for endogenous and non-synaptic properties. *J Neurophysiol* **75**: 124–132.
- PATRYLO PR, COPUS AJ, SCHWEITZER JS, DUDEK FE, 1996. Multiple-unit recordings during slow field-potential shifts in low  $[Ca^{2+}]_o$  solutions in rat hippocampal and cortical slices. *Neuroscience* **74**: 107–118.
- PATRYLO PR, SCHWEITZER J, DUDEK FE, 1994. Potassium-dependent prolonged field bursts in the dentate gyrus: Effects of extracellular calcium and amino acid receptor antagonists. *Neuroscience* **61**: 13–19.
- PEREZ-VALAZQUEZ JL, VALIANTE TA, CARLEN PL, 1994. Modulation of gap junctional mechanisms during calcium-free induced field burst activity: a possible role for electronic coupling in epileptogenesis. *J Neurosci* **14**: 4308–4317.
- RASH JE, DUFFY HS, DUDEK FE, BILHARTZ BL, WHALEN LR, YASUMURA T, 1997. Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a 'pan-glial syncytium' that is not coupled to neurons. *J Comp Neurol* **388**: 265–292.
- RASH JE, DILLMAN RK, MORITA M, WHALEN LR, GUTHRIE PB, FAY-GUTHRIE D, WHEELER DW, 1995. Grid-mapped freeze fracture: Correlative confocal laser scanning microscopy and freeze-fracture electron microscopy of preselected cells in tissue slices. In: Severs NJ, Shotton DM, eds. *Rapid Freezing, Freeze Fracture, and Deep Etching*. New York, Wiley-Liss, Inc. 127–150.
- RASH JE, DILLMAN RK, BILHARTZ BL, DUFFY HS, WHALEN LR, YASUMURA T, 1996. Mixed synapses discovered and mapped throughout mammalian spinal cord. *Proc Natl Acad Sci USA* **93**: 4235–4239.
- RASH JE, YASUMURA T, NIELSEN S, AGRE P, HUDSON CS, RASH JL, 1998. Contributions of freeze fracture to neurobiology: High-resolution replication and immunogold labeling of connexons and aquaporins in grid-mapped replicas of rat brain and spinal cord. *Proceedings of the XIVth International Congress for Electron Microscopy* (submitted).
- RICHARDSON TL, O'REILLY CO, 1995. Epileptiform activity in the dentate gyrus during low-calcium perfusion and exposure to transient electric fields. *J Neurophysiol* **74**: 388–399.
- ROPER SN, OBENAU A, DUDEK FE, 1992. Lowered osmolality causes non-synaptic epileptiform bursts in rat dentate gyrus: a comparison with area CA1. *Ann Neurol* **31**: 81–85.
- ROPER SN, OBENAU A, DUDEK FE, 1993. Increased propensity for nonsynaptic epileptiform activity in immature rat hippocampus and dentate gyrus. *J Neurophysiol* **70**: 857–862.
- RORIG B, SUTOR B, 1996. Regulation of gap junction coupling in the developing neocortex. *Molec Neurobiol* **12**: 225–249.

- SCHMALBRUCH H, JAHNSEN H, 1981. Gap junctions on CA3 pyramidal cells of guinea pig hippocampus shown by freeze-fracture. *Brain Res* **217**: 175–178.
- SCHNEIDER SP, 1992. Demonstration of artifactual coupling between spinal neurons and glial cells during intracellular recording with micropipette electrodes. *Brain Res* **585**: 343–348.
- SCHWARTZKROIN PA, PRINCE DA, 1980. Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. *Brain Res* **183**: 61–76.
- SCHWEITZER JS, PATRYLO PR, DUDEK FE, 1992. Prolonged field bursts in the dentate gyrus: dependence on low calcium, high potassium and nonsynaptic mechanisms. *J Neurophysiol* **68**: 2016–2025.
- SLOPER JJ, 1972. Gap junctions between dendrites in the primate neocortex. *Brain Res* **44**: 641–646.
- SLOPER JJ, POWELL TPS, 1978. Gap junctions between dendrites and somata of neurons in the primate sensori-motor cortex. *Proc R Soc Lond (Biol)* **203**: 39–47.
- SNOW RW, DUDEK FE, 1984. Synchronous epileptiform bursts without chemical transmission in CA2, CA3 and dentate areas of the hippocampus. *Brain Res* **298**: 382–385.
- SPENCER WA, KANDEL ER, 1961. Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *J Neurophysiol* **24**: 272–285.
- STRATA F, ATZORI M, MOLNAR M, UGOLINI G, TEMPIA F, CHERUBINI E, 1997. A pacemaker current in dye-coupled hilar interneurons contributes to the generation of giant GABAergic potentials in developing hippocampus. *J Neurosci* **17**: 1435–1446.
- STUART GJ, SAKMANN B, 1994. Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature* **367**: 69–72.
- STUART G, SCHILLER J, SAKMANN B, 1997a. Action potential initiation and propagation in rat neocortical pyramidal neurons. *J Physiol* **505**: 617–632.
- STUART G, SPRUSTON N, SAKMANN B, HAUSSER M, 1997b. Action potential initiation and backpropagation in neurons of the mammalian CSN. *Trend Neurosci* **20**: 125–131.
- TAYLOR CP, DUDEK FE, 1982a. A physiological test for electrotonic coupling between CA1 pyramidal cells in rat hippocampal slices. *Brain Res* **235**: 351–357.
- TAYLOR CP, DUDEK FE, 1982b. Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. *Science* **218**: 810–812.
- TAYLOR CP, DUDEK FE, 1984a. Excitation of hippocampal pyramidal cells by an electrical field effect. *J Neurophysiol* **52**: 126–142.
- TAYLOR CP, DUDEK FE, 1984b. Synchronization without active chemical synapses during hippocampal afterdischarges. *J Neurophysiol* **52**: 143–155.
- TRAUB RD, 1995. Model of synchronized population bursts in electrically coupled interneurons containing active dendritic conductances. *J Comput Neurosci* **2**: 283–289.
- TRAUB RD, WONG RKS, 1982. Cellular mechanism of neuronal synchronization in epilepsy. *Science* **216**: 745–747.
- TRAUB RD, DUDEK FE, TAYLOR CP, KNOWLES WD, 1985a. Simulation of hippocampal afterdischarges synchronized by electrical interactions. *Neuroscience* **14**: 1033–1038.
- TRAUB RD, DUDEK FE, SNOW RW, KNOWLES WD, 1985b. Computer simulations indicate that electrical field effects contribute to the shape of the epileptiform field potential. *Neuroscience* **15**: 947–958.
- TRAUB RD, SCHMITZ D, JEFFERYS JGR, DRAGUHN A, 1999. High frequency population oscillations are predicted to occur in hippocampal pyramidal neuronal networks interconnected by axoaxonal gap junctions. *Neuroscience* **92**: 407–426.
- TRAYNELIS SF, DINGLELINE R, 1988. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J Neurophysiol* **59**: 259–276.
- TRAYNELIS SF, DINGLELINE R, 1989. Role of extracellular space in hyperosmotic suppression of potassium-induced electrographic seizures. *J Neurophysiol* **61**: 927–938.
- VALIANTE TA, PEREZ VELAZQUEZ JL, JAHROMI SS, CARLEN PL, 1995. Coupling potentials in CA1 neurons during calcium-free-induced field burst activity. *J Neurosci* **15**: 6946–6956.
- VIGMOND EJ, PEREZ-VELAZQUEZ JL, VALIANTE TA, BARDAKJIAN BL, CARLEN PL, 1997. Mechanisms of electrical coupling between pyramidal cells. *J Neurophysiol* **78**: 3107–3116.
- WONG RKS, PRINCE DA, BASBAUM AI, 1979. Intradendritic recordings from hippocampal neurons. *Proc Natl Acad Sci USA* **76**: 986–990.
- YAARI Y, KONNERTH A, HEINEMANN U, 1986. Nonsynaptic epileptogenesis in the mammalian hippocampus *in vitro*. II. Role of extracellular potassium. *J Neurophysiol* **56**: 424–438.
- YUSTE R, NELSON DA, RUBIN WW, KATZ LC, 1995. Neuronal domains in developing neocortex: mechanisms of coactivation. *Neuron* **14**: 7–17.