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Inhibition, Local Excitatory Interactions and Synchronization of Epileptiform Activity in Hippocampal Slices

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Inhibition counteracts local excitatory influences between hippocampal neurons in the normal brain, thus maintaining independent channels for information processing. Removal of synaptic inhibition has provided the most widely employed experimental model for epileptogenesis. Inhibitory mechanisms reduce the efficacy of excitatory chemical synapses, thereby increasing the probability of transmission failure through local excitatory pathways. After removal of inhibition, local excitatory chemical synapses recruit increasing numbers of pyramidal cells in a positive feedback manner. Differences in the epileptogenicity of various areas of the hippocampus (e.g., CA3 versus CA1), when synaptic inhibition is blocked, may be due largely to the density and strength of recurrent excitation. Evidence is also available for electrotonic coupling and electrical field effects among hippocampal neurons. Electrical mechanisms can synchronize hippocampal neurons when chemical synapses are blocked with low-$[Ca^{2+}]$ solutions. Removal of inhibition would also be expected to strengthen electrical interactions, although less is known about them. Electrical interactions probably combine with local chemical excitation to synchronize neurons when inhibition is compromised.

Epileptogenesis is thought to involve alterations in the balance of excitatory and inhibitory synaptic interactions. Recurrent excitation via chemical synapses synchronizes the depolarization shifts associated with epileptiform bursts observed after blockade of inhibitory circuits, but electrical interactions can also synchronize neurons (see Dudek, Snow, and Taylor, 1986 for review). Recent studies, which will be reviewed here, have provided new insights into how both excitatory synaptic and electrical mechanisms may interact to

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promote the transition from normal to epileptiform activity after pharmacological blockade of inhibition.

Dual intracellular recordings are the most direct way to analyze local neuronal interactions. Similarly, synchronization and its underlying mechanisms are probably best studied with simultaneous intracellular and extracellular recordings. In hippocampal slices, these methods have produced evidence for local interactions via both chemical synapses (inhibitory and excitatory) and electrical mechanisms. The contributions of local neuronal interactions (chemical and electrical) to epileptogenesis have also been assessed in the hippocampus with the above techniques.

In this chapter we will present experimental results that deal with the following questions: Does inhibition suppress local excitatory interactions? Does blockade of inhibition allow multi-synaptic interactions and "reverberating circuits"? Finally, how may chemical synapses and electrical interactions combine to synchronize neurons during epileptiform bursting? We will focus first on chemical synaptic mechanisms, and then on electrical interactions.

Methods and Results

Recurrent Excitation Through Chemical Synapses

Monosynaptic interactions in CA3. Several lines of evidence, primarily obtained with extracellular stimulation and intracellular recording, initially suggested a prominent role for recurrent synaptic excitation in the synchronization and spread of epileptiform activity (e.g., see Ayala, Dichter, Gumnit, Matsumoto, and Spencer, 1973). Later, dual intracellular recordings provided direct evidence that both inhibitory and excitatory interactions were present in the CA3 area of rat hippocampal slices (MacVicar and Dudek, 1980). A single action potential from an intracellular current pulse in one hippocampal pyramidal cell evoked an excitatory response in other pyramidal cells (5 of 88 cell pairs, approximately 6%). The short latency of the responses and the low occurence of transmission failures in 4 of the 5 pairs suggested that the connections were monosynaptic (see Figure 1A). These experiments also implied that each pyramidal cell only projects to a small fraction of the nearby pyramidal cells (MacVicar and Dudek, 1980). Subsequent theoretical studies, based on these and other data, suggested that very few excitatory synaptic interactions were required for synchronization of epileptiform bursts (Traub and Wong, 1982). Recent experiments with dual intracellular recordings in the CA3 area of guinea pig hippocampal slices have confirmed these findings in that they also showed monosynaptic excitatory interactions (see Figure 1B) between a very low percentage of the cell pairs (i.e., 7 of 400, approximately 2%) [Miles and Wong, 1986b].

Blockade of inhibition reveals polysynaptic excitation in CA3. Two types of studies concerning the effects of synaptic inhibition on local excitatory interactions have recently been undertaken; Miles and Wong (1986a, 1986b) have used dual intracellular recordings, while our work has employed glutamate microapplication as a method for stimulating hippocampal neurons

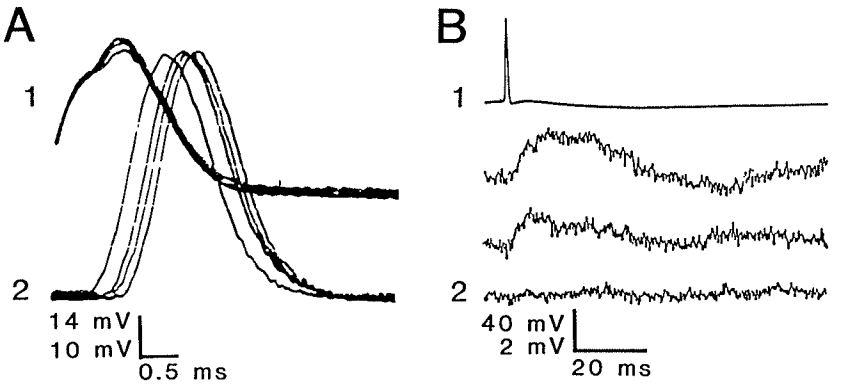

Figure 1: Monosynaptic interactions between CA3 pyramidal cells revealed with dual intracellular recording. A single action potential was evoked in a pyramidal cell (Trace 1), and postsynaptic responses were recorded intracellularly from another nearby pyramidal cell (Trace 2). (A) Superimposed traces showing that each presynaptic spike evoked a postsynaptic action potential in another pyramidal cell. The unidirectional nature and short latency of the response suggest that the two cells were connected by a chemical synapse (from MacVicar and Dudek, 1980). Reprinted by permission from *Brain Research, 184*, 220–223, Copyright © 1980, Elsevier Science Publishers. (B) An action potential in one cell evoked EPSPs in a nearby CA3 pyramidal cell. The three lower traces show two EPSPs and one synaptic failure (from Miles and Wong, 1986b). Reprinted by permission from *The Journal of Physiology, 373*, 397–418, Copyright © 1986, The Physiological Society.

(i.e., pyramidal cells and interneurons) independent of extrinsic afferents (e.g., see Goodchild, Dampney, and Bandler, 1982). In our experiments, a CA3 pyramidal cell was impaled with an intracellular micropipette containing QX-314 to block Na^+-mediated action potentials (Connors and Prince, 1982), and another micropipette was used to pressure-apply glutamate microdrops (10–20 mM) to the surface of the slice (see Figure 2, inset). We confirmed with several control experiments that glutamate does not directly activate the mossy fiber axons, which innervate the CA3 pyramidal cell bodies and proximal dendrites. Furthermore, tetrodotoxin blocked glutamate-induced increases in the frequency of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs); these PSPs were therefore interpreted to result from stimulation of local circuits, rather than axons and presynaptic terminals.

We applied glutamate microdrops both before and after treatment with 5–10 μM picrotoxin (Christian and Dudek, 1988a). The extracellular fluid contained a high concentration of divalent cations (i.e., $[Ca^{2+}]$ and $[Mg^{2+}]$ were

4mM) to raise threshold and reduce picrotoxin-induced epileptiform bursting. Prior to picrotoxin, a microdrop of glutamate applied to the slice surface in the CA3 stratum pyramidale usually caused an increase in the frequency of IPSPs, with little or no detectable change in EPSP frequency (see Figure 2A). After picrotoxin was added to the medium, a similar glutamate microdrop often significantly increased EPSP frequency (see Figure 2B). The data imply that under normal conditions, inhibition suppresses activity within local excitatory circuits. Conversely, these excitatory interactions are unmasked when inhibition is blocked.

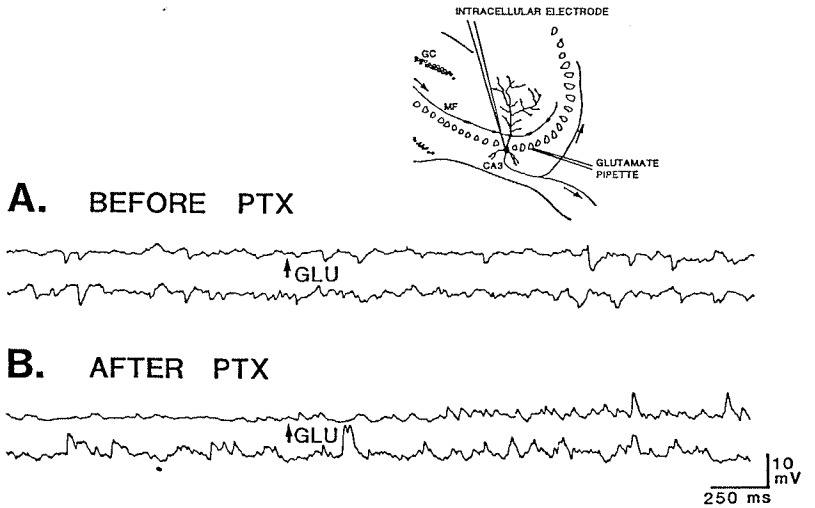

Figure 2: Effects of picrotoxin on local inhibitory and excitatory interactions studied with glutamate microstimulation. Inset shows recording configuration; an intracellular electrode recorded from a CA3 pyramidal cell, and a glutamate-containing micropipette was positioned approximately 200 μm away from the impaled cell to activate local circuits independent of extrinsic axons. (A) Before perfusion with picrotoxin, a glutamate microdrop (arrow) caused an increase in IPSP frequency. (B) After perfusion with a picrotoxin-containing solution, a similar glutamate microdrop applied to the same location caused a pronounced increase in EPSP frequency for 5–10 sec (Christian and Dudek, 1988a). Reprinted by permission from Chalazonitis and Gola (Eds.), *Inactivation of Hypersensitive Neurons*, 147–156, Copyright © 1987, Alan R. Liss, Inc.

Miles and Wong (1986a) have undertaken experiments with dual intracellular recording in picrotoxin-treated slices. Although only 2% of the pairs of simultaneously impaled CA3 pyramidal cells showed monosynaptic excitatory interactions in normal solution (see above), blockade of inhibition by 5 μM picrotoxin revealed evidence for excitatory interactions between 7 of 21 paired recordings (i.e., 33%). These interactions showed longer synaptic delays and a much larger percentage of synaptic failures, which would be expected of polysynaptic interactions (see Figure 3). Their data imply that blockade of inhibition facilitates extensive multineuronal synaptic

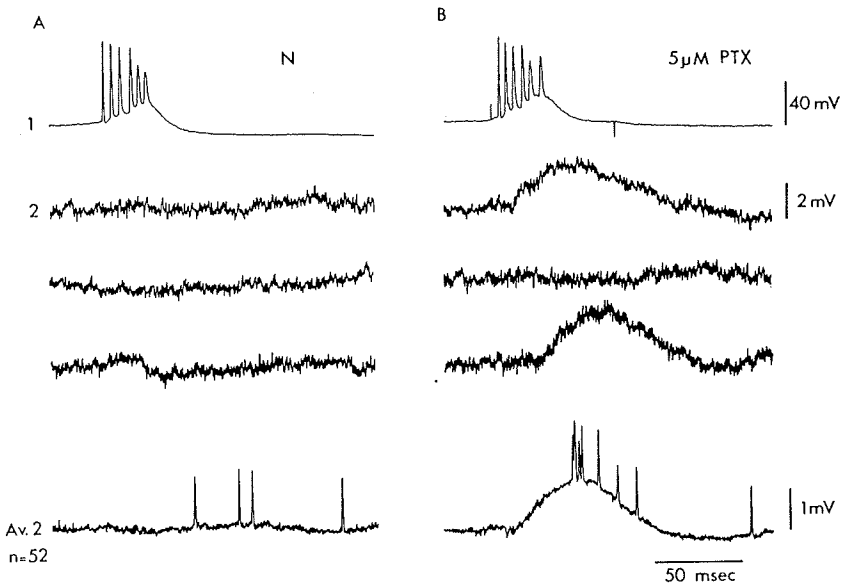

Figure 3: Effects of picrotoxin on excitatory interactions between hippocampal pyramidal cells revealed with dual intracellular recording. The upper trace (Trace 1) in both A and B shows a spike burst in one CA3 pyramidal cell and the lower traces illustrate the postsynaptic responses from a different cell. The three middle traces are individual responses, and the lowest trace is an average of 52 sweeps. (A) In a solution with picrotoxin, no EPSPs were evoked. (B) The individual traces indicate that long-latency EPSPs were occasionally evoked in 5 μM picrotoxin. The lowest trace shows an averaged excitatory synaptic response (Miles and Wong, 1986a). Reprinted by permission of R. Miles and R.K.S. Wong (unpublished data).

transmission through local excitatory circuits. Conversely, inhibition tends to prevent divergent excitatory synaptic activity between nearby cells. Miles and Wong (1983) also found that stimulation of a single CA3 pyramidal cell in a picrotoxin-treated slice could occasionally evoke synchronous population bursts (see Figure 4), which strongly supports the hypothesis of a positive feedback mechanism through divergent local excitatory circuits (i.e., a "cascade" effect).

Local excitatory circuits and the transition to epileptiform bursting. Before synchronous bursting occurred in the CA3 area of picrotoxin-treated slices, Miles and Wong (1986a) observed an apparent transition period during which the frequency and amplitude of spontaneously occuring EPSPs were increased. The number of synchronous EPSPs in dual recordings was also increased. Although our studies were performed in solutions with high concentrations of divalent cations to lower excitability and raise the threshold for epileptiform bursting, we found prolonged afterdischarges of large EPSPs after glutamate microdrops (see Figure 5) in seven cells from isolated fragments of CA3

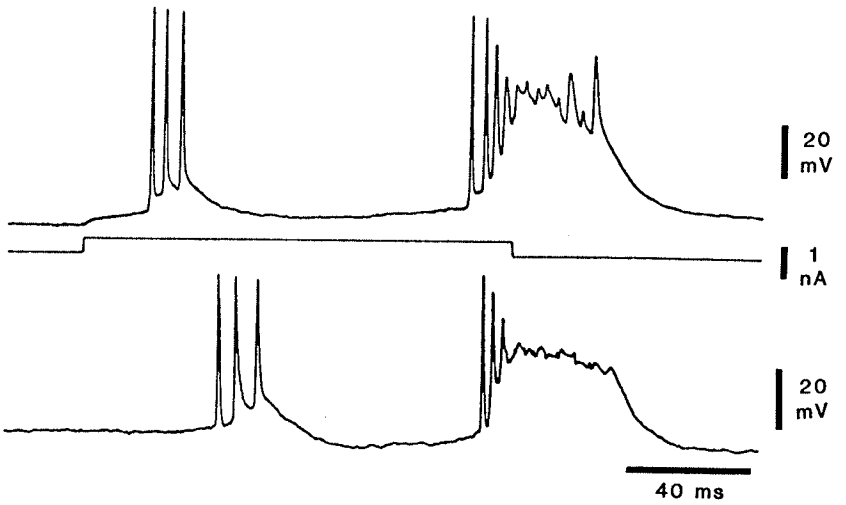

Figure 4: Stimulation of a single CA3 pyramidal cell can evoke a synchronous burst in a picrotoxin-treated slice. The upper trace shows a spike burst evoked with an intracellular current pulse (middle trace). The evoked spike burst triggered a long-latency burst in another pyramidal cell (lower trace), which then led to a synchronous burst of the neuronal population (from Miles and Wong, 1983). Reprinted by permission from *Nature*, 306, 371–373, Copyright © 1983, Macmillan Magazines, Limited.

(Christian and Dudek, 1988a). The effects of a glutamate microdrop normally only lasted 5–10 sec (see Figure 2); in these seven cells, however, the afterdischarges of large EPSPs could persist for up to 2 minutes. Epileptiform bursting was eventually observed in two of the seven cells that showed these long afterdischarges of EPSPs. Therefore, both research groups have observed a transitional period of hyperexcitability that appears to be manifested by a pronounced activation of local excitatory synapses; in some of our experiments, the afterdischarges of large EPSPs observed with glutamate microstimulation could reflect "reverberating" activity through these excitatory synapses, which may be an intermediate phase preceding epileptiform bursting. These studies are also consistent with previous data indicating that a particularly large synaptic potential, presumably from recurrent excitatory synapses, causes the depolarization shifts associated with epileptiform bursting in CA3 pyramidal cells (Johnston and Brown, 1981).

Local excitatory circuits in CA1. The CA3 area has been considered much more susceptible than CA1 to epileptiform bursting when synaptic inhibition is pharmacologically reduced with penicillin (Schwartzkroin and Prince, 1978). In 100 pairs of dual intracellular recordings, Knowles and Schwartzkroin (1981) observed inhibitory interactions (13%) but no synaptic excitation between CA1 pyramidal cells. However, in later studies by Hablitz (1984), isolated fragments of CA1 in picrotoxin-treated slices were clearly shown to generate epileptiform activity, but synchronous bursting was observed less frequently in

CA1 than in CA3. Although electrical mechanisms could have synchronized the bursts (see below), the data of Hablitz (1984) suggested that recurrent excitation exists among CA1 pyramidal cells. We therefore undertook experiements using glutamate microstimulation in isolated CA1 fragments from picrotoxin-treated slices (Christian and Dudek, 1988b), similar to those

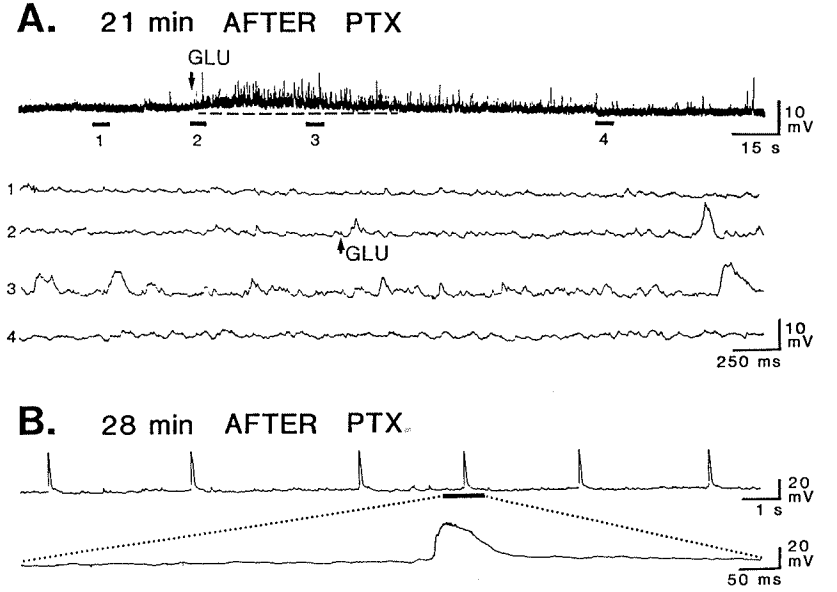

Figure 5: Afterdischarges of glutamate-evoked EPSPs after picrotoxin treatment. The recording configuration and experimental procedures were similar to those illustrated in Figure 2. (A) In some picrotoxin-treated slices, a glutamate microdrop evoked a prolonged afterdischarge of large-amplitude EPSPs lasting up to 2 min. (B) Two cases of epileptiform bursting were observed, and they followed the long afterdischarges seen in A (Christian and Dudek, 1988a). Reprinted by permission from Chalazonitis and Gola (Eds.), *Inactivation of Hypersensitive Neurons*, 147–156, Copyright © 1987, Alan R. Liss, Inc.

described above in CA3. Since the experiments of Knowles and Schwartzkroin (1981) were performed in longitudinal slices and those of Hablitz (1984) involved transverse slices, we compared local excitatory circuits in both slice orientations. We found that glutamate microdrops could evoke increases in EPSP frequency in both slice orientations (see Figure 6); however, local excitatory circuits appeared to be less dense in the longitudinal orientation than in the transverse direction, and CA1 had less local excitation than CA3. Furthermore, under identical conditions of picrotoxin treatment (including high concentrations of divalent cations), neither long afterdischarges of EPSPs nor epileptiform bursts were ever observed in CA1. These data suggest that although local inhibitory circuits are readily demonstrated with dual intracellular recording in CA1, a sparse system of local excitatory circuits is difficult to detect with this technique, particularly when inhibition is present.

Furthermore, the propensity for epileptiform activity when synaptic inhibition is reduced or blocked appears to depend, at least partially, on the characteristics of local excitatory circuits.

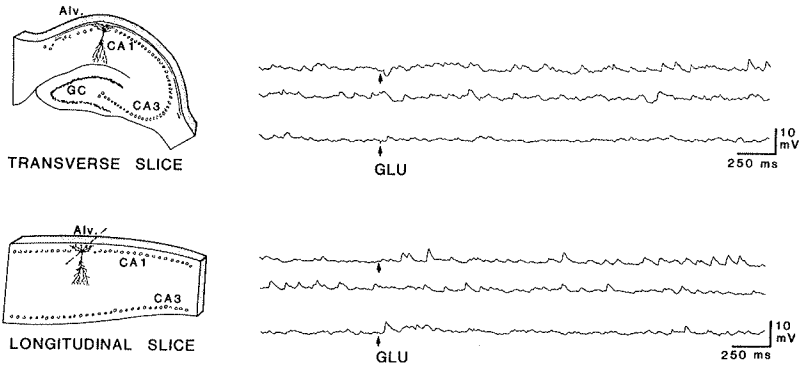

Figure 6: Excitatory interactions in the isolated CA1 area of picrotoxin-treated slices. In transverse slices, glutamate microdrops could evoke increases in EPSP frequency at some locations (top two traces), but not others (bottom trace). Similar but less frequent increases were also seen in longitudinal slices. The increases in EPSP frequency in longitudinal slices (top two traces) were usually only observed when glutamate microdrops were applied close to the cell but not at longer distances (bottom trace). The top two traces for both the transverse and longitudinal slices are continuous (modified from Christian and Dudek, 1988b).

Electrical Interactions

Although a variety of experimental and theoretical studies have suggested that chemical synaptic mechanisms are important for synchronizing the depolarization shifts observed in penicillin- or picrotoxin-treated slices (e.g., Johnston and Brown, 1981; Traub and Wong, 1982), chemical synaptic mechanisms probably cannot account for the fast synchronization required to produce hippocampal population spikes (see below).

Experiments from several laboratories have now shown that hippocampal neurons (i.e., CA1 and CA3 pyramidal cells, and also dentate granule cells) can fire synchronous bursts of population spikes when chemical synaptic mechanisms are blocked with low-[Ca^{2+}] solutions (Snow and Dudek, 1984a). Previous evidence for electrotonic coupling via gap junctions and electrical field effects (i.e., ephaptic interactions) has been reviewed recently (Dudek et al., 1986) and thus will receive less consideration. Both types of electrical interaction could hypothetically provide a fast mechanism of cell interaction capable of synchronizing action potentials.

Electrotonic coupling. Several lines of evidence from electrophysiological experiments, intracellular tracer injections and ultrastructural observations support the hypothesis that some hippocampal pyramidal and dentate granule cells are electronically coupled to neighboring cells via gap junctions (Dudek,

Andrew, MacVicar, Snow, and Taylor, 1983; Dudek et al., 1986). However, it is unclear how many hippocampal neurons are actually coupled. MacVicar and Jahnsen (1985) have recently shown that exposure of hippocampal slices to propionate, which would be expected to acidify the intracellular space and reduce gap junctional conductance, causes a significant reduction in the percentage of CA3 pyramidal cells that are dye-coupled. This recent observation adds strong support to the hypothesis of electrotonic coupling through neuronal gap junctions in hippocampus. Inhibitory synaptic input would tend to shunt electrotonic junctions (e.g., for discussion, see Llinas, Baker and Sotelo, 1974; MacVicar and Dudek, 1981; Spira and Bennett, 1972). Conversely, blockade of inhibition would be expected to enhance transmission through electrotonic junctions by removing a shunt of non-junctional membrane. It is noteworthy that fast prepotentials, which are traditionally considered to represent dendritic spikes, are more prominent in penicillin-treated slices (Schwartzkroin and Prince, 1980); however, these events could also be electrotonic coupling potentials that are augmented when tonic inhibitory mechanisms are removed (see MacVicar and Dudek [1981] for discussion). Further experiments are necessary to resolve this issue. Nonetheless, synaptic inhibition probably reduces the efficacy of both electrical and chemical synapses.

Electrical field effects. Differential transmembrane recordings (i.e., intracellular minus extracellular), under conditions where chemical synaptic transmission was blocked, have revealed electrical field effects (i.e., ephaptic transmission) between CA1 pyramidal cells during hippocampal population spikes (e.g., Taylor and Dudek, 1982, 1984a, 1984b). Epileptiform bursts in picrotoxin-treated slices are also often associated with large population spikes. When these cells were hyperpolarized with steady injected currents to block action potentials (or injected with QX-314), differential recordings of transmembrane potential revealed field effect depolarizations on the peak of the depolarization shifts (see Figure 7). These field effect depolarizations were correlated with and appear to be caused by the population spikes observed in extracellular recordings (Snow and Dudek, 1984b). Other data suggest that field effect depolarizations can occur in normal solutions throughout the hippocampus (i.e., CA1 and CA3) and dentate gyrus whenever population spikes are present (Snow and Dudek, 1986). Field effects to antidromic population spikes have also been observed in situ (Taylor, Krnjević, and Ropert, 1984; Yim, Krnjević, and Dalkara, 1986), and recent data suggest that acetylcholine and bicuculline promote the synchronizing and excitatory effects of ephaptic currents (Dalkara, Krnjević, Ropert, and Yim, 1986). Finally, computer models provide strong theoretical support for the hypothesis that field effects contribute significantly to synchronization in low-$[Ca^{2+}]$ media (Traub, Dudek, Taylor, and Knowles, 1985) and after treatment with picrotoxin (Traub, Dudek, Snow, and Knowles, 1985).

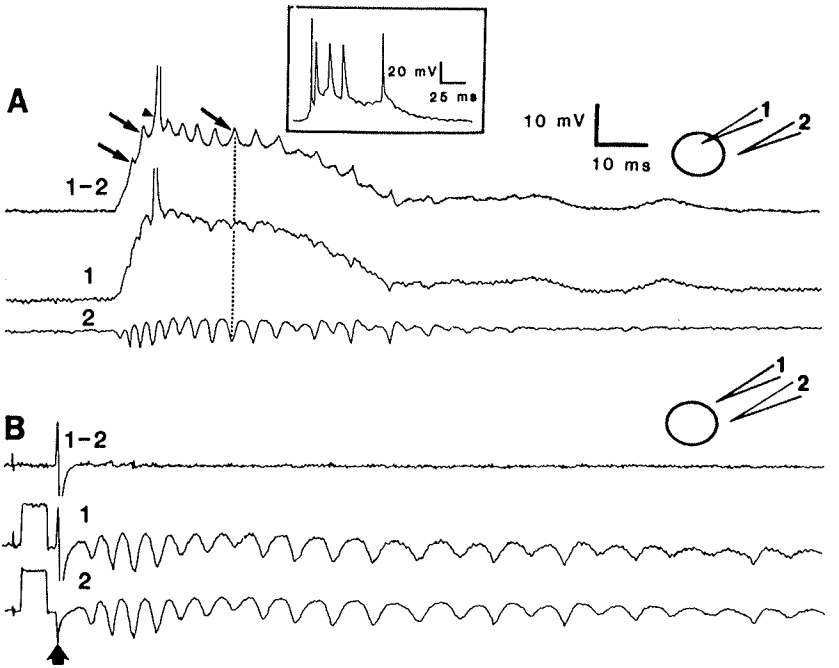

Figure 7: Electrical field effects during picrotoxin-induced bursting. Inset shows example of a spontaneous spike burst superimposed on a depolarization shift in an intracellularly recorded hippocampal neuron. (A) Differential recording from a CA3 pyramidal cell (see inset), which was hyperpolarized with a steady injected current to block action potentials, revealed field effect depolarizations (see arrows). At the peak of the depolarization shift (arrowhead), a field effect depolarization appeared to evoke an action potential. Note that the field effect depolarizations were synchronous with the population spikes recorded extracellularly (dotted line). The single-ended intracellular recording (trace 1) only revealed brief negativities during the population spikes. (B) Control differential recordings (trace 1–2) showed a flat baseline when the intracellular electrode was withdrawn from the impaled cell. The similarity of the recordings (traces 1 and 2) under these conditions indicate that electrode 2 recorded the actual extracellular potential during the spike burst in A (Snow and Dudek, 1984b). Reprinted by permission from Delgado-Escueta, Ward, Woodbury, and Porter (Eds.), *Basic Mechanisms of the Epilepsies: Molecular and Cellular Approaches*, 593–617, Copyright © 1986, Raven Press, New York.

Discussion

Inhibitory Effects on Local Excitatory Interactions

Chemical synapses. It has long been realized that inhibition tends to shunt or obscure synaptic excitation from afferent stimulation (e.g., Dingledine and Gjerstad, 1983; Schwartzkroin and Prince, 1980), and so it is not surprising that inhibition also obscures *local* excitatory synapses. For chemical synaptic

mechanisms, each cell in CA3 is capable of exciting a few neighboring cells, which can then excite many others. The presence of inhibition appears to block this positive feedback process mediated by divergent excitatory pathways. Independent of electrical mechanisms (see below), the balance between local excitatory and inhibitory circuits appears to be critical.

Electrotonic coupling. A very similar effect of synaptic inhibition presumably also modulates the efficacy of electrotonic coupling. Even though several lines of evidence support the presence of coupling in the hippocampus and elsewhere in the mammalian brain, the same data also suggest that some hippocampal neurons are not coupled and that the coupled cells are only connected in isolated groups or clusters. Coupling alone appears to be too sparse to synchronize cells when inhibition is blocked, but an enhanced degree of coupling after dendrotomy (Gutnick, Lobel-Yaakov, and Rimon, 1985) or other treatments might lead to an increased contribution when inhibition is reduced.

Electrical field effects. As with chemical and electrical synaptic interactions, a decrease in inhibition will tend to increase the strength of electrical field effects (see Taylor and Dudek [1984a] for discussion, also Dalkara et al. [1986]). Somatic shunts from synaptic inhibition would reduce ephaptic interactions. Furthermore, electrical field effects would tend to augment the synchronization derived from the synaptic mechanisms outlined above, particularly in hyperexcitable tissue when spike threshold is low. Even population spikes that are only in the millivolt range appear to have excitatory influences on nearby inactive cells (Krnjević, Dalkara, and Yim, 1986; Taylor and Dudek, 1984a).

Therefore, several possible mechanisms for excitatory interaction are present in the hippocampus, and all of them can lead to positive feedback. Each excitatory mechanism is probably enhanced when inhibition is blocked. Since strong recurrent inhibitory pathways exist in the hippocampus, all increases in activity would normally be counterbalanced by enhancement of inhibitory tone. Feed-forward inhibitory pathways would serve as a projection mechanism for altering inhibitory tone and thus changing the likelihood of synchronous activity.

Similarities and Differences in Hippocampal Subfields

The available data indicate that an extensive inhibitory network is present throughout all areas of the hippocampus. Several lines of evidence, however, suggest differences in local excitatory mechanisms between the various hippocampal subfields. Inhibitory synaptic mechanisms tend to block all local excitatory interactions; when inhibition is decreased, the electrical behavior of a neuronal population depends on the local excitatory mechanisms (chemical and electrical) which are present in that region of the hippocampus. Loss of

inhibitory synapses (e.g., after picrotoxin treatment) versus loss of *both* inhibitory and local excitatory synapses (e.g., low-$[Ca^{2+}]$ solutions) would be expected to have different effects on the collective behavior of the various hippocampal populations.

Local excitatory pathways mediated by chemical synapses appear to be more extensive in the CA3 area than in CA1 (see above), and undetectable in the dentate gyrus. When inhibition *alone* is blocked, the CA3 area is more susceptible than CA1 to epileptiform activity, and both of these areas are more likely to fire epileptiform bursts than the dentate gyrus. This is probably due in large part to the density of recurrent excitatory circuits mediated by chemical synapses, although intrinsic conductances probably also contribute.

No clear and consistent difference across hippocampal neuronal populations has been reported with regard to electrotonic coupling, but this form of interaction is difficult to assess quantitatively and therefore differences might be hard to detect (e.g., Dudek et al., 1983). If the amplitude of field potentials is used as a rough estimate for the strength of electrical field effects, then ephaptic transmission is probably more powerful in the CA1 area and the dentate gyrus than in CA3. Although field effects are clearly present in the CA3 area (Snow and Dudek, 1984a, 1986; Taylor et al., 1984), they may be less powerful because of the lower packing density of these neurons. When both inhibitory *and* excitatory chemical synapses are blocked, the more densely packed areas (CA1 and dentate gyrus) are more susceptible to epileptiform bursting than the CA3 area; intrinsic conductance mechanisms are presumably involved here also.

Inhibition and Epilepsy

The role of synaptic inhibition in human epilepsy remains unclear. There is no doubt, however, that removal of inhibition is one dramatic way to cause epileptiform activity in animal models. Transient hypoxia may preferentially damage inhibitory circuits (Dunwiddie, 1981), and thus increase the propensity for epileptogenesis. Increases in extracellular $[K^+]$, in addition to depolarizing neurons, appear to indirectly reduce synaptic inhibition (see Korn, Giacchino, Chamberlin, and Dingledine, 1987; Rutecki, Lebeda, and Johnston, 1985). Alterations in the level of inhibitory tone presumably occur throughout the normal brain, but reductions below some critical level would be expected to cause epileptiform activity. From the statements made above, one important contributing factor to the susceptibility of an area to epileptogenic activity would be the type and strength of local excitatory interactions in that area. Intense electrical activity would be expected to activate recurrent inhibitory circuits repetitively. A high level of activity in these recurrent inhibitory circuits might eventually cause them to become depressed, similar to the phenomenon of GABA "fading" (Ben-Ari, Krnjević, Reiffenstein, and

Reinhardt, 1981, which would then lead to epileptogenesis.

Several of the excitatory mechanisms of local neuronal interaction may have synergistic effects, particularly when inhibition is depressed. For example, during the period with increased amplitude and frequency of EPSPs and possible reverberating activity (see above), it is possible that both chemical and electrical mechanisms act together to enhance synchronization. Small groups of neurons, possibly synchronized by electrical interactions, may fire simultaneously to generate large multi-component EPSPs in a postsynaptic cell that receives convergent input from recurrent chemical synapses. Further work is needed on the mechanisms by which inhibition alters local excitatory interactions, and on precisely how decreases in inhibition affect the collective or integrative properties of hippocampal neuronal populations.

Possible Roles for Inhibition in the Normal Brain.

Although we have focused on how alterations in inhibition may lead to epileptiform activity, inhibition is known to be a prominent feature of many areas of the normal brain, and it is presumably of fundamental importance in neuronal integration. Numerous other areas of the brain besides the hippocampus are known to have one or more mechanisms of local excitatory interaction. In vitro studies of epileptogenesis have revealed insights into how neurons might recruit other nearby cells, or begin to synchronize them, when inhibitory mechanisms are reduced. Each of these local mechanisms appears to act at a different time scale; that is, ionic and chemical synaptic mechanisms are slower than the electrical interactions, which can act nearly instantaneously. Inhibition would be expected to desynchronize cells when the general tone is increased, and it tends to make individual and population firing patterns more variable. Roberts (1986) has provided a general statement on how inhibition in the brain would tend to desynchronize cells and increase the variability in their group firing patterns. Just as smooth movement requires that motoneurons fire asynchronously, it is possible that the degree of asynchrony in neuronal firing is important in the normal function of the hippocampus. However, relatively little is known about how local inhibitory circuits are activated during normal function in the brain.

References

Ayala, G.F., Dichter, M., Gumnit, R.J., Matsumoto, H., and Spencer, W.A. (1973). Genesis of epileptic interictal spikes. New knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms. *Brain Research*, 52, 1–17.

Ben-Ari, Y., Krnjević, K., Reiffenstein, R.J., and Reinhardt, W. (1981). Inhibitory conductance changes and action of γ-aminobutyrate in rat hippocampus. *Neuroscience*, 6, 2445–2463.

Christian, E.P., and Dudek, F.E., (1983a). Characteristics of local excitatory circuits studied with glutamate microapplication in the CA3 area of rat hippocampal slices. *Journal of Neurophysiology*. (in press)

Christian, E.P., and Dudek, F.E. (1988b). Electrophysiological evidence from glutamate microapplications for local excitatory circuits in the CA1 area of rat hippocampal slices. *Journal of Neurophysiology.* (in press)

Connors, B.W., and Prince, D.A. (1982). Effects of local anesthetic QX-314 on the membrane properties of hippocampal pyramidal neurons. *Journal of Pharmacology and Experimental Therapeutics, 220,* 476–481.

Dalkara, T., Krnjević, K., Ropert, N., and Yim, C.Y. (1986). Chemical modulation of ephaptic activation of CA3 hippocampal pyramids. *Neuroscience 17,* 361–370.

Dingledine, R., and Gjerstad, L. (1983). Penicillin blocks hippocampal IPSPs, unmasking prolonged EPSP's. *Brain Research, 168,* 205–209.

Dudek, F.E., Andrew R.D., MacVicar, B.A., Snow, R.W., and Taylor, C.P. (1983). Recent evidence for and possible significance of gap junctions and electrotonic synapses in the mammalian brain. In H.H. Jasper and N.M. van Gelder (Eds.), *Basic mechanisms of neuronal hyperexcitability* (pp. 31-73). New York: Alan R. Liss.

Dudek, F.E., Snow, R.W., and Taylor, C.P. (1986). Role of electrical interactions in synchronization of epileptiform bursts. In A.V. Delgado-Escueta, A.A. Ward, Jr., D.M. Woodbury, and R.J. Porter (Eds.), *Basic mechanisms of the epilepsies: Vol. 44, Advances in Neurology* (pp. 593–617). New York: Raven Press.

Dunwiddie, T.V. (1981). Age-related differences in the *in vitro* rat hippocampus. Development of inhibition and the effects of hypoxia. *Developmental Neuroscience, 4,* 165–175.

Goodchild, A.K., Dampney, R.A.L., and Bandler, R. (1982). A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system. *Journal of Neuroscience Methods 6,* 351–363.

Gutnick, M.J., Lobel-Yaakov, R., and Rimon, G. (1985). Incidence of neuronal dye-coupling in neocortical slices depends on the plane of section. *Neuroscience, 15,* 659–666.

Hablitz, J.J. (1984). Picrotoxin-induced epileptiform activity in hippocampus: Role of endogenous versus synaptic factors. *Journal of Neurophysiology, 51,* 1011–1027.

Johnston, D., and Brown, T.H. (1981). Giant synaptic potential hypothesis for epileptiform activity. *Science, 211,* 294–297.

Knowles, W.D., and Schwartzkroin, P.A. (1981). Local circuit synaptic interactions in hippocampal brain slices. *Journal of Neuroscience, 1,* 318–322.

Korn, S.J., Giacchino, J.L., Chamberlin, N.L., and Dingledine, R. (1987). Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *Journal of Neurophysiology, 57* 325–340.

Krnjević, K., Dalkara, T., and Yim, C. (1986). Synchronization of pyramidal cell firing by ephaptic currents in hippocampus *in situ.* In R. Schwarcz and Y. Ben-Ari (Eds.), *Excitatory amino acids and epilepsy* (pp. 413–423). New York: Plenum Press.

Llinas, R., Baker, R., and Sotelo, C. (1974). Electrotonic coupling between neurons in cat inferior olive. *Journal of Neurophysiology, 37,* 560–571.

MacVicar, B.A., and Dudek, F.E. (1980). Local synaptic circuits in rat hippocampus: Interactions between pyramidal cells. *Brain Research, 184,* 220–223.

MacVicar, B.A., and Dudek, F.E. (1981). Electrotonic coupling between pyramidal cells: A direct demonstration in rat hippocampal slices. *Science, 213,* 782–785.

MacVicar, B.A., and Jahnsen, H. (1985). Uncoupling of CA3 pyramidal neurons by propionate. *Brain Research, 330,* 141–145.

Miles, R., and Wong, R.K.S. (1983). Single neurons can intitiate synchronized population discharge in the hippocampus. *Nature, 306,* 371–373.

Miles, R., and Wong, R.K.S. (1986a). Excitatory synaptic connexions between guinea-pig CA3 hippocampal cells are revealed when synaptic inhibition is suppressed *in vitro. Journal of Physiology, 372,* 14P.

Miles, R., and Wong, R.K.S. (1986b). Excitatory synaptic interactions between CA3 neurons in the guinea-pig hippocampus. *Journal of Physiology, 373,* 397–418.

Roberts, E. (1986). What do GABA neurons really do? They make possible variability generation in relation to demand. *Experimental Neurology, 93,* 279–290.

Rutecki, P.A., Lebeda, F.J., and Johnston, D. (1985). Epileptiform activity induced by changes in extracellular potassium in hippocampus. *Journal of Neurophysiology, 54,* 1363–1374.

Schwartzkroin, P.A., and Prince, D.A. (1978). Cellular and field potential properties of epileptogenic hippocampal slices. *Brain Research, 147,* 117–130.

Schwartzkroin, P.A., and Prince, D.A. (1980). Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. *Brain Research, 183*, 61–76.

Snow, R.W., and Dudek, F.E. (1984a). Synchronous epileptiform bursts without chemical transmission in CA2, CA3 and dentate areas of the hippocampus. *Brain Research, 298*, 382–385.

Snow, R.W., and Dudek, F.E. (1984b). Electrical fields directly contribute to action potential synchronization during convulsant-induced epileptiform bursts. *Brain Research, 323*, 114–118.

Snow, R.W., and Dudek, F.E. (1986). Evidence for neuronal interactions by an electrical field effect in CA3 and dentate regions of rat hippocampal slices. *Brain Research, 367*, 292–295.

Spira, M.E., and Bennett, M.V.L. (1972). Synaptic control of electrotonic coupling between neurons. *Brain Research, 37*, 294–300.

Taylor, C.P., and Dudek, F.E. (1982). Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. *Science, 218*, 810–812.

Taylor, C.P., and Dudek, F.E. (1984a). Excitation of hippocampal pyramidal cells by an electrical field effect. *Journal of Neurophysiology, 52*, 126–142.

Taylor, C.P., and Dudek, F.E. (1984b). Synchronization without active chemical synapses during hippocampal afterdischarges. *Journal of Neurophysiology, 52*, 143–155.

Taylor, C.P., Krnjević, K., and Ropert, N. (1984). Facilitation of hippocampal CA3 pyramidal cell firing by electrical fields generated antidromically. *Neuroscience, 11*, 101–109.

Traub, R.D., and Wong, R.K.S. (1982). Cellular mechanism of neuronal synchronization in epilepsy. *Science, 216*, 745–747.

Traub, R.D., Dudek, F.E., Snow, R.W., and Knowles, W.D. (1985). Computer simulations indicate that electrical field effects contribute to the shape of the epileptiform field potential. *Neuroscience, 15*, 947–958.

Traub, R.D., Dudek, F.E., Taylor, C.P., and Knowles, W.D. (1985). Simulation of hippocampal afterdischarges synchronized by electrical interactions. *Neuroscience, 14*, 1033–1038.

Yim, C.C., Krnjević, K., and Dalkara, T. (1986). Ephaptically generated potentials in CA1 neurons of rat's hippocampus *in situ*. *Journal of Neurophysiology, 56*, 99–122.