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Interneurones are not so dormant in temporal lobe epilepsy: a critical reappraisal of the dormant basket cell hypothesis

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Abstract

One axiom at the basis of epilepsy research is that there exists an imbalance between excitation and inhibition. This abnormality can be achieved by an increase of excitation on principal cells, a decreased inhibition (i.e. disinhibition) or both. This review focuses on dysfunction of inhibition, and in particular on the 'dormant basket cell hypothesis'. This hypothesis states that, (1) interneurones are functionally disconnected from excitatory afferents, resulting in hyperexcitability of principal neurones and loss of paired pulse inhibition, (2) when properly activated, interneurones can still perform their task, i.e. suppress epileptiform activity and restore paired pulse inhibition. The aim of this review is to discuss the evidence in support of the 'dormant basket cell hypothesis'. We will first discuss the rationale underlying the hypothesis and the criteria needed to validate the hypothesis. We will then show that, (1) the key experimental data offered in support of the hypothesis (Bekenstein and Lothman, 1993. Dormancy of inhibitory interneurones in a model of temporal lobe epilepsy. Science 259, 97–100; Sloviter, 1991. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the 'dormant basket cell' hypothesis and its relevance to temporal lobe epilepsy. Hippocampus 1, 41–66) are difficult to interpret, and (2) recent recordings from interneurones in epileptic tissue argue against the hypothesis. The 'dormant basket cell hypothesis' is then discussed in the broader context of disinhibition.

Keywords: Dormant basket cell hypothesis; Interneurones; Epilepsy; Disinhibition

1. Foreword

In 1877, Claude Bernard wrote about the Principles of Applied Medicine: 'The experimental method is nothing but bringing observation and experiment into operation in order to get access to scientific truth. Some use the results of observation and experiment to build theories that they no longer put to test. They could be called a priorists. Instead, one's inferences are to be tested by new experiments. This is the true experimental method everyone knows about and which M. Cheuvreul calls the a posteriori experimental method. However, this is not yet sufficient. Even when attempting to verify one's inference by an experiment or

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an observation, it is necessary to remain the slave of the observation, as well as of the experiment. One must not be overcome by one's inductive idea that is nothing but a hypothesis. I can say that I follow such a precept. Thus, the verification of my inferring hypothesis, whatever its likelihood, does not blind me. I hold conditionally to it. Therefore, I am trying as much to invalidate as to verify my hypothesis. In short, I do research with an open mind. This is the reason why I so often found results I was not looking for while investigating other things I could not find. The truth must be the goal of our studies. Being satisfied by plausibility or likelihood is the true pitfall.'

2. Introduction

In the hippocampus, the neuronal population can be divided into two broad classes: principal neurones which are excitatory and use glutamate as a neurotransmitter, and interneurones which are inhibitory and use γ-amino butyric acid (GABA) as a neurotransmitter (Freund and Buzsáki, 1996). In normal tissue, principal neurones, which represent 90% of the neuronal population, are under the control of interneurones. The latter provide constant background GABAergic activity that drives the membrane potential of principal neurones away from firing threshold. In epileptic tissue, principal neurones are usually hyperexcitable and three non exclusive pathophysiological mechanisms are thought to underlie this hyperexcitability: (1) modifications in membrane properties, (2) increased excitatory drive (Tauck and Nadler, 1985; Represa et al., 1987; Lanerolle et al., 1989; Represa et al., 1989; Represa and Ben-Ari, 1992; Sundstrom et al., 1993; Perez et al., 1996), and (3) decreased inhibitory drive or disinhibition. Research on epilepsy has essentially focused on the latter concept since: (1) drugs boosting fast GABAergic inhibition have been used successfully to treat certain forms of epilepsy, including temporal lobe epilepsy (TLE), (2) pharmacological blockade of fast GABAergic inhibition is epileptogenic (Prince, 1978), and (3) fast GABAergic inhibition collapses during high frequency activity (Thompson and Gahwiler, 1989). There are five testable propositions which would support disinhibition as a key contributor to epileptiform activity: (1) the death of some populations of interneurones, (2) a functional disconnection of the latter from their excitatory afferents, (3) a downregulation of their firing properties, (4) a modification at the presynaptic level resulting in a decreased GABA release, (5) postsynaptic changes resulting in a decreased hyperpolarisation or shunting effect. The 'dormant basket cell hypothesis' (dbch) specifically addresses the second of these possible pathophysiological mechanisms of disinhibition. This hypothesis has been proposed as a way to reconcile apparent contradictory observations: the structural basis of the inhibitory system remains relatively intact in epileptic tissue but there is a loss of synaptic inhibition and paired-pulse inhibition. In this review, we will address three issues: (1) what is the dormant basket cell hypothesis? (2) has it been experimentally supported? and (3) how can it be verified? The hypothesis is then discussed in the broader concept of disinhibition.

Since there is some confusion about the formulation of the dbch (Bekenstein and Lothman, 1993), it is first necessary to recall Sloviter's own definition.

3. What is the dormant basket cell hypothesis?

As stated by Sloviter (1991b), the dbch 'implies that although seizure-resistant GABA neurones survive, they are hypofunctional because they have lost critical excitatory inputs' and that 'a reversible loss of inhibition can occur in experimental tissues in which most GABA neurones appear, at least morphologically, to be relatively normal'. Thus, the dbch contains TWO aspects that cannot be dissociated: 'the loss of excitatory systems that normally evoke inhibition' and 'the reversibility of lost inhibition'. As a consequence, the dbch is not merely a disconnection theory.

Since this hypothesis has been so influential, it seems appropriate to step back and analyze the premises on which it is based.

In the animal model of TLE used by Sloviter (1987), immunocytochemical data showed an irre-

versible damage of dentate hilar mossy cells that were believed to provide excitatory inputs onto interneurones and of somatostatin-immunoreactive interneurones. In contrast, most dentate gyrus GABA-immunoreactive basket cells were preserved (Sloviter, 1987), a feature also reported in the CA1 area of the hippocampus (Sloviter, 1991b). Further studies suggested that inhibitory interneurones were also spared in human (Babb et al., 1989). Since the inhibitory network appeared morphologically intact, Sloviter proposed that 'the decreased inhibition and epileptiform behavior that occurred after initial stimulation might be the result of damage to cells that normally excite inhibitory interneurones', i.e. mossy cells.

There are thus three premises: (1) the general preservation of the inhibitory network, (2) the loss of excitatory afferents onto GABAergic interneurones, and (3) decreased inhibition on principal cells (disinhibition). However, the following results call into question the integrity of the GABAergic neurones, their disconnection from excitatory afferents and the concept of disinhibition:

(1) The inhibitory network is not intact. Some GABAergic interneurones do degenerate in human TLE (DeLanerolle et al., 1989; Robbins et al., 1991; Mathern et al., 1995; Marco et al., 1996) and chronic animal models of TLE (Ben-Ari et al., 1987; Sloviter, 1987; Obenaus et al., 1993; Best et al., 1994; Houser and Esclapez, 1996). Indeed, most ($>90\%$) of the somatostatin-immunoreactive neurones, which are largely destroyed in TLE, are GABAergic (Somogyi et al., 1984; Esclapez and Houser, 1994).

(2) The concept that major excitatory inputs to interneurones are lost in TLE (Sloviter, 1987, 1991b) is not supported by the following morphological data. Mossy cells (that degenerate in TLE) provide only a small percentage of excitatory afferents onto basket cells. Most of the glutamatergic inputs to interneurones (basket cells) originate from the entorhinal cortex, from granule cells (mossy fiber collaterals), and from CA3 pyramidal cells (Ribak and Seress, 1983; Zipp et al., 1989; Seress and Ribak, 1990a,b; Ribak and Peterson, 1991; Kneisler and Dingledine, 1995a,b). In particular, the granule cells of the fascia dentata do not degenerate in TLE and seem to provide a powerful excitatory drive onto hilar interneurones (Acsady et al., 1997).

(3) The dbch is embedded in the more general concept of disinhibition, which is supposed to be a fundamental feature of epilepsy. However, recent reports have challenged this concept (Prince et al., 1997).

(i) In chronic animal models of epilepsy in the neocortex, both anatomical and electrophysiological markers of inhibition are increased (Prince et al., 1997); nevertheless, pyramidal cells are hyperexcitable. This apparent paradox clearly indicates that the relationship between epileptogenesis and disinhibition is not straightforward. As pointed out by Prince et al. (1997), 'successful treatment of human epilepsy with GABAergic drugs does not imply an underlying defect in inhibitory electrogenesis'. Similarly, the fact that $GABA_A$ receptor antagonists are convulsivant does not make the loss of inhibition a necessary condition for epileptogenesis.

(ii) In the dentate gyrus of many animal models of TLE, inhibition is enhanced. Paired pulse inhibition is more powerful (Haas et al., 1996) and the $GABA_A$ receptor current density is increased by 100% (Gibbs et al., 1997).

(iii) In the CA1 area of the hippocampus in such animal models, there is a 50% decrease of the conductance of the $GABA_A$ receptor current density (Gibbs et al., 1997), but inhibition is still operative in the soma of pyramidal cells (Esclapez et al., 1997). Inhibition is, however, markedly decreased in the apical dendrites (Bernard et al., 1997).

All these studies lead to the conclusion that inhibition is either increased or affected at the level of the GABAergic synapses on the principal cell.

Thus, the first aspect of the premises of the dbch theory, the preservation of interneurones, is not completely valid. The third aspect—decreased inhibition in principal cells—is also not clearly supported. The second aspect, the disconnection of interneurones from excitatory inputs, is not supported in 'absolute' terms, but only in relative terms. Indeed, in most chronic models of TLE (kindling, kainic acid, pilocarpine), there is a loss

of some principal neurones, in particular of CA3 pyramidal cells (Nadler et al., 1978; Sloviter, 1987; Obenaus et al., 1993). As a result, there is a loss of Schaffer collaterals and/or commissural fibers that provide a major excitatory input to CA1 GABAergic interneurones (Freund and Buzsáki, 1996). The fate of excitatory inputs to dentate GABAergic interneurones is more difficult to assess. Like mossy cells, we also know that some CA3 pyramidal cells, which project back to the hilus (Ishizuka et al., 1990; Li et al., 1994; Kneisler and Dingledine, 1995b), degenerate. Thus, there will be a loss of glutamatergic drive on interneurones, including dentate gyrus basket cells. We can therefore conclude that a loss of excitatory synapses on hippocampal interneurones is more than likely. However, several fundamentally important issues remain to be addressed:

(1) The quantification of the loss of glutamatergic synapses (e.g. at the electron microscopic level).

(2) The specificity of this loss in relation to subpopulations of interneurones.

(3) The compensation of this loss by a reactive synaptogenesis as is known to occur in CA1 pyramidal cells or dentate granule cells (Tauck and Nadler, 1985; Represa et al., 1987; Lanerolle et al., 1989; Represa et al., 1989; Represa and Ben-Ari, 1992; Sundstrom et al., 1993; Perez et al., 1996).

(4) The functional outcome of such a loss on the activity of interneurones. The decreased excitatory drive could well be compensated by a modification of the membrane properties of the interneurones, leading to an increase in excitability and/or by a decreased inhibitory control by the population of interneurone-specific interneurones (Freund and Buzsáki, 1996).

4. Loss of functional inhibition

Let us assume that interneurones have lost a critical set of excitatory afferents. What data support the view that this loss results in dysfunction of inhibition and hyperexcitability in principal cells?

In a subsequent electrophysiological study, Sloviter (1991b) examined the predictions of this disconnection theory in dentate granule cells and CA1 pyramidal neurones. Extracellular recordings of population spikes were obtained in an in vivo kindling model of TLE. Measurement of population spikes (PS) in CA1 stratum pyramidale and stratum granulosum following ipsilateral stimulation of the perforant path (PP) suggested a loss of paired pulse inhibition and hyperexcitability in both types of principal neurones. However, when the PP was activated contralaterally to the kindled side, paired pulse inhibition was restored in CA1 pyramidal cells (Sloviter, 1991b). Sloviter then concluded that interneurones, having lost critical excitatory inputs ipsilaterally, cannot perform their task when activated through this pathway, but can still be activated via a surviving pathway which provides efficient and functional inhibition. Unfortunately, as stated by Sloviter (1991b), it was not determined if it was possible 'to evoke inhibition in the damaged dentate gyrus by activating the commissural projection from surviving mossy cells on the unstimulated side'. Thus, it is unclear if there is generalizeable support for this restoration-of-inhibition aspect of the dbch.

In these studies, the criteria to evaluate the loss and the recovery of functional inhibition were the failure (or the presence) of paired pulse inhibition (Sloviter, 1991a,b). Several features of the reported results make it difficult to interpret these data. The concerns focus on the experimental paradigm.

(1) The absence of quantitative data in both studies (Sloviter, 1991a,b) precludes the comparison between control and epileptic tissue. Interpretations were based on visual inspection of the records.

(2) The loss of paired pulse inhibition could be due to the use of low intensity stimulation; increasing the stimulus intensity restores paired pulse inhibition (Kapur et al., 1989).

(3) Low frequency stimulation (2–3 Hz), used to activate CA1 pyramidal cells (Sloviter, 1991a,b), produces short term modifications of PS amplitudes (Bernard and Wheal, 1995) via a modification of the firing threshold of pyramidal cells (Bernard and Wheal, 1996) as well as a transient

failure of paired pulse inhibition (Bernard and Wheal, unpublished observations). Indeed, the population spikes evoked by 2–3 Hz stimulation appear to reflect a combination of multiple factors, including a failure of inhibition (see Figs. 7A2, B2 and C2 in Sloviter (1991a)) and epileptiform activity (see Figs. 7B2, C2 and D in Sloviter (1991a)), which make them difficult to interpret.

(4) In the dentate gyrus, it has been repeatedly reported that inhibition is increased in vitro, in various models of TLE, in the time window used by Sloviter (1991a,b), i.e. 2–6 weeks after status epilepticus. In particular, when very low frequency stimulation (0.05 Hz) is used, paired pulse inhibition is very powerful (Haas et al., 1996). In contrast, at higher frequencies (2–3 Hz) paired pulse inhibition is lost and epileptiform discharges may appear (see Fig. 2C 2–3 in Sloviter (1991a)).

In conclusion, the frequency of stimulation (and possibly its intensity) used by Sloviter (1991a,b) may have triggered numerous dynamical processes affecting the strength of inhibition. The mechanisms are still too complex to interpret and the experiments do not prove the dbch. However, the experimental data are consistent with the hypothesis, for when the surviving pathway was activated paired pulse inhibition was restored (Figs. 5B4 and 5C4 in (Sloviter, 1991b)). As stated by Sloviter (1991b), 'tentative conclusions drawn from these results form only a working hypothesis for future intracellular studies'. Such a study appeared 2 years later (Bekenstein and Lothman, 1993).

5. Do the results provided by Bekenstein and Lothman demonstrate that inhibitory interneurones are dormant?

Intracellular recordings of CA1 pyramidal cells were performed in vitro in a different kindling model of TLE (Bekenstein and Lothman, 1993). If the slice tissue was stimulated 'far' from the site of the recording electrode, IPSPs were not seen. However, when stimulations were applied closer to the recording site, IPSPs could be measured in the presence of glutamatergic antagonists. These data were interpreted to support the concept of functional disconnection of interneurones from their excitatory inputs—a disconnection that could be overcome by more direct stimulation of the interneurones. However, the experimental paradigm used does not allow an accurate measure of IPSP for the following reasons.

(1) The conductance and reversal potential of IPSPs were not measured. A change in these parameters would have affected the 'visibility' of the IPSP. In fact, the conductance of $GABA_A$ receptor current density is decreased by 50% in the pilocarpine model of TLE (Gibbs et al., 1997).

(2) The duration of the evoked response could have masked the IPSP. Indeed, IPSPs are not seen underlying suprathreshold responses because they are masked by the presence of a depolarizing after potential (see Fig. 2B in Bekenstein and Lothman (1993)), the duration of the discharge and/or the activation of NMDA receptors (Hirsch et al., 1996; Mangan and Lothman, 1996). Moreover, subthreshold stimulation produces an EPSP/IPSP sequence (see Fig. 3 in Franck et al. (1988)). Therefore, the main argument of Bekenstein and Lothman's study—the apparent absence of IPSPs when far orthodromic stimulations were used—is not clearly supported by their data. In fact, when adequate experimental protocols are used, i.e. voltage clamp recordings at the reversal potential of glutamatergic currents, large multiphasic GABAergic currents are measured during (Esclapez et al., 1997; Prince et al., 1997) and between (Esclapez et al., 1997) spontaneous and evoked epileptiform discharges in CA1 pyramidal cells.

(3) Although pharmacologically isolated IPSPs could still be measured in CA1 pyramidal cells with 'near' stimulation, such recordings mainly provide information about postsynaptic inhibition, not about the fate of circuitry-mediated inhibition. In the presence of glutamatergic antagonists, the network loses its most prominent feature, its epileptogenicity.

Because IPSPs were not 'seen' in the absence of glutamatergic antagonist, Bekenstein and Lothman concluded that 'basket cells are disconnected irrespective of the routes by which inhibitory interneurones are excited' (Bekenstein and Lothman, 1993). However, the best way to prove that

basket cells are fully disconnected is to record from basket cells themselves, not from pyramidal cells. Importantly, if basket cells were indeed fully disconnected, the dbch could not be true for the hypothesis assumes the existence of alternative excitatory pathways, which, when activated, would excite basket cells and thus restore functional inhibition.

6. What should be done to prove the dbch?

Since the hypothesis contains two parts, each of them should be addressed.

(1) First, the dbch proposes that some interneurones have lost a critical set of excitatory afferents. This could occur via an anatomical loss of excitatory synapses and/or a decrease of their response (long-term depression of synaptic responses, postsynaptic downregulation of AMPA/NMDA receptors, declustering, etc.). These modifications should affect more the interneurones than the principal cells, so that interneurones have a lower probability of discharge in TLE than in control when the critical afferent pathway is stimulated. The best way to address this issue is to perform simultaneous recordings of a presynaptic neurone (whose axon belongs to the critical excitatory pathway) and its targets (interneurone and principal cell). Electrophysiological responses and anatomical properties (morphology and electron microscopy) could then be compared between interneurone and principal cell, in control and TLE.

(2) Second, the dbch suggests the possibility of 'revival' of inhibition. Again, the best way to test this part of the hypothesis is to record simultaneously from interneurones and principal cells in vivo. According to the dbch, the stimulation of the PP ipsilaterally to the 'lesion' should not activate a subpopulation of interneurones, whereas a contralateral stimulation should activate them. This pattern should be paralleled by a failure and a reactivation of paired pulse inhibition, respectively, in the principal cell. This condition holds true even if we take the occurrence of epileptiform discharges instead of the failure of paired pulse inhibition as an index of disinhibition. It should then be demonstrated that the same interneurone population that is disconnected is also involved in paired pulse inhibition. Such a subpopulation of interneurones could involve more than the basket cell type originally proposed.

These experiments are technically feasible, although very difficult to perform since they require dual recordings in vivo. The use of the slice for such experiments is precluded since, in this preparation, the Schaffer collateral and commissural pathways cannot be activated independently. A new preparation which allows in vitro recordings in both intact hippocampal formations should prove useful (Khalilov et al., 1997). However, to examine responses of interneurones to afferent stimulation, the slice preparation can yield important results; direct recordings from interneurones have been obtained in the slice preparation in chronic models of TLE.

7. What do the recordings of interneurones in chronic models of TLE tell us?

So far, five studies have reported the recording of interneurones in the CA1 area in chronic animal models of TLE (Franck et al., 1988; Nakajima et al., 1991; Morin et al., 1996; Esclapez et al., 1997; Rempe et al., 1997).

(1) In the bilateral kainic acid (KA) model of TLE, five putative interneurones were recorded 2–4 months after KA injection, and ten neurones 2–4 weeks after the lesion (four of which were morphologically identified post hoc as interneurones) (Franck et al., 1988). All five cells recorded at 2–4 months responded to stimulation with an EPSP or an action potential. At 2–4 weeks, eight out of the ten putative interneurones responded with EPSPs or action potentials. It was concluded 'that the interneurones present following KA were functional at the same time when CA1 was hyperexcitable'.

(2) In a subsequent study from the same group (Nakajima et al., 1991), six putative basket cells were recorded simultaneously with pyramidal cells, 2–4 weeks after the bilateral KA lesion. Four out of six interneurones responded with a

burst of action potentials and the other two with an EPSP in response to stratum radiatum stimulation. All pyramidal cells fired bursts of action potentials.

(3) In the same model, evoked EPSCs were measured in stratum oriens (SO, $n = 10$), stratum radiatum (SR, $n = 10$) and lacunosum moleculare (LM, $n = 10$) interneurones (recorded under visual control) and compared to those measured in similar populations of interneurones in control (Morin et al., 1996). The amplitudes of EPSCs were found to be identical in control and TLE, except in LM interneurones for which the amplitudes of the EPSCs were decreased by 50% in TLE.

(4) A more systematic study was performed in a kindling model of TLE (Rempe et al., 1997). Interneurones were recorded under visual control in SR-LM ($n = 27$ in TLE, $n = 35$ in control) and SO ($n = 13$ in TLE, $n = 19$ in control). The intrinsic properties of these interneurones, the characteristics of evoked EPSCs, the spontaneous glutamatergic synaptic activity and the paired pulse facilitation of EPSCs were identical in TLE and control. Interneurones were found more hyperexcitable in TLE than in control.

(5) In the KA and pilocarpine models of TLE (Esclapez et al., 1997), interneurones were recorded in SO ($n = 2$), stratum pyramidale ($n = 5$), SR ($n = 7$) and LM ($n = 2$). The axonal arborisation of these interneurones was characterized post hoc. Six perisomatic projecting interneurones (basket cells or chandelier cells) were identified (four in stratum pyramidale, two in SR). Stimulation of their excitatory afferents evoked epileptiform discharges in 14 out of 16 interneurones (including all putative basket cells), the remaining two responding with a single action potential. Spontaneous and evoked glutamatergic epileptiform discharges were also seen in three pairs of CA1 pyramidal cell/perisomatic interneurone recorded simultaneously.

8. Are these results consistent with the two components of the dbch?

(1) Functional disconnection of interneurones from a critical set of excitatory afferents. None of these studies directly addressed this issue. However, known morphological facts allow some speculation. In all these animal models of TLE (except for the pilocarpine model), there is a massive loss of CA3 pyramidal cells ipsilaterally to the lesion (Nadler et al., 1978; Sloviter, 1987). Thus, the resulting loss of Schaffer collaterals, which provide a major excitatory input to interneurones (Freund and Buzsáki, 1996), should result in a deafferentation visible at the electron microscopic level, as demonstrated for CA1 pyramidal cells (Nadler et al., 1980a,b; Phelps et al., 1991). Nevertheless, evoked synaptic responses in interneurones and pyramidal cells are identical in TLE and control (Morin et al., 1996; Rempe et al., 1997). Therefore, it can be proposed that the reactive synaptogenesis that occurs in pyramidal cell dendrites (Phelps et al., 1991) also affects interneurones. Alternatively, the expected decreased excitatory drive could have been compensated by presynaptic (increased probability of release or vesicle content) or postsynaptic (long-term potentiation) modifications of the surviving synapses. Thus, even if interneurones have lost a critical set of afferents, there are compensatory mechanisms, yet to be determined, which apparently support their activation as in control (as least as seen in vitro).

So far, interneurones and their response to stimulations in stratum radiatum have been recorded in the CA1 area in vitro. Such stimulation does not allow the separation of the different excitatory pathways, since commissural fibers are co-mingled with Schaffer collaterals (Gottlieb and Cowan, 1973; Laurberg, 1979; Chan, 1992). Nevertheless, 94% (59 out of 63) of the interneurones recorded with sharp electrodes (Franck et al., 1988; Nakajima et al., 1991), in the cell attached configuration (Esclapez et al., 1997) or in current clamp mode (Esclapez et al., 1997; Rempe et al., 1997), fired either one or a burst of action potentials following stimulation in stratum radiatum. The four interneurones that failed to fire an action potential or to respond with an EPSP (Franck et al., 1988; Nakajima et al., 1991) were recorded before the animals had developed spon-

taneous recurrent seizures; such failures were not found in chronically epileptic animals (Franck et al., 1988; Rempe et al., 1997). The same distinction applies to LM interneurones for which the amplitudes of the evoked EPSCs were decreased by 50% shortly after the lesion (Morin et al., 1996), a feature not found in chronically epileptic animals (Rempe et al., 1997). Finally, the absence of evoked EPSP could be explained by the shunting effect and/or the large driving force of $GABA_A$ receptor-mediated responses—this is a classical feature described in pyramidal cells, where IPSPs are often evoked without apparent EPSP. Thus, even if there is a functional disconnection of a few interneurones, it is but a marginal (and apparently transitory) phenomenon.

(2) Revival of inhibition. As pointed out previously, this issue needs to be addressed in vivo. However, recordings of interneurones give us interesting hints about this aspect of the hypothesis. A direct prediction of the dbch is that when a surviving pathway (i.e. the commissural pathway) is stimulated, interneurones fire action potentials and restore functional inhibition. Since commissural fibers and Schaffer collaterals have overlapping patterns of connectivity, in vitro stimulations also activated the surviving pathway, i.e. the commissural fibers. This factor could explain why in vitro stimulation of CA1 striatum radiatum resulted in the firing of almost all interneurones (Esclapez et al., 1997; Rempe et al., 1997), including basket cells (Esclapez et al., 1997) in chronically epileptic animals. Despite the firing of interneurones, paired pulse inhibition failed and pyramidal cells were hyperexcitable (Esclapez et al., 1997). This result is not consistent with the view that activation of commissural afferents restores inhibition.

Another result arguing against the dbch comes from studies investigating the amount of inhibition necessary to keep neuronal networks 'under control'. In contrast to the neocortex where a 10% reduction of inhibition is sufficient for epileptogenesis (Chagnac-Amitai and Connors, 1989), over 80% of inhibition must be antagonized to generate epileptiform activity in the CA1 region of the hippocampus (Jefferys and Whittington, 1996). This result suggests that most interneurones should be dormant according to the dbch of TLE—which is clearly not the case.

Of course, it is possible that, in recording from interneurones in TLE, all previous investigators systematically missed the specific subpopulation of interneurones that is primarily responsible for inhibition in hippocampus, and that controls paired pulse inhibition and hyperexcitability in CA1 pyramidal cells. The existence of such a population of interneurones seems improbable; if such a population does exist, it does not include all basket cells.

In conclusion, although none of the studies described above directly addressed the functional disconnection of interneurones from a critical set of excitatory afferents, this hypothetized deafferentation does not seem to affect interneuron function (Esclapez et al., 1997; Rempe et al., 1997). On the contrary, interneurones in models of TLE appear more hyperexcitable than in control (Esclapez et al., 1997; Rempe et al., 1997). These studies appear to invalidate a strict disconnection theory (Bekenstein and Lothman, 1993) and certainly do not provide support for the dbch.

9. The dbch and the concept of disinhibition

As stated previously, the general concept of disinhibition does not seem to be a fundamental feature of epilepsy. Many recent studies lead to the conclusion that inhibition is either increased or unaffected at the level of the GABAergic synapses on principal cells. That is, if inhibition is decreased, it does not involve a downregulation of interneurone function. Thus, the fate of inhibition in epileptic tissue becomes a highly complex issue for which a straightforward and unique answer seems unlikely.

(1) It is area specific. It may depend upon the animal model used, the preparation (in vivo versus in vitro) and more importantly the time of investigation after the lesion. Epilepsy being a dynamical process, the various measurable parameters are likely to change following status epilepticus, during the relapse period or after the establishment of recurrent seizures (Kapur and Macdonald, 1997).

(2) The neuronal tissue is characterized by a high degree of plasticity. Various compensatory phenomena can occur. They include: (i) the sprouting of the axonal arbor of interneurones (Seil et al., 1994), (ii) the sprouting of excitatory fibers onto interneurones (such as occurs onto principal cells) (Tauck and Nadler, 1985; Represa et al., 1987; Lanerolle et al., 1989; Represa and Ben-Ari, 1992; Sundstrom et al., 1993; Perez et al., 1996), (iii) a decreased inhibitory drive mediated by a population of interneurone-specific interneurones (Freund and Buzsáki, 1996), (iv) an upregulation of the excitability of the interneurones (Esclapez et al., 1997; Rempe et al., 1997), or (v) a facilitation of inhibition at the level of the GABAergic synapse (Gibbs et al., 1997).

(3) Several features of disinhibition remain to be investigated. They include (i) the functional consequence of the death of specific sub-populations of interneurones (Ben-Ari et al., 1987; Sloviter, 1987; DeLanerolle et al., 1989; Robbins et al., 1991; Obenaus et al., 1993; Best et al., 1994; Mathern et al., 1995; Marco et al., 1996) or loss of specific GABAergic synapses, (ii) failure of GABA release, (iii) depletion of transmitter from the presynaptic terminals, (iv) activation of inhibitory autoreceptors, and (v) downregulation of the receptors by Ca^{2+} or other compounds.

10. Conclusion

(1) Disinhibition per se does not seem critical for epileptogenesis, especially given that compensatory mechanisms may result in enhanced inhibition. Thus, the concept of dormant interneurones as an explanation for epileptiform activity is certainly not universally applicable.

(2) The initial experiments addressing the dbch are difficult to interpret because of the complexities of the experimental paradigms.

(3) If the interneurones recorded by various research groups are representative of the whole population of inhibitory cells in the CA1 area, then the direct recordings of interneurones demonstrate that they are not functionally disconnected from their excitatory afferents. On the contrary, they appear to be more hyperexcitable in models of TLE than in controls. Moreover, their activation does not restore paired pulse inhibition and does not prevent the occurrence of epileptiform discharges in CA1 pyramidal cells, as forecast by the dbch.

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References

Acsady, L., Kamondi, A., Sik, A., Hirase, H., Freund, T.F., Buzsáki, G., 1997. Synaptic targets of hippocampal granule cells: an in vivo intracellular labeling study. Soc. Neurosci. Abstr. 23, 483.

Babb, T., Pretorius, J., Kupfer, W., Crandall, P., 1989. Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. J. Neurosci. 9, 2562–2574.

Bekenstein, J., Lothman, E., 1993. Dormancy of inhibitory interneurons in a model of temporal lobe epilepsy. Science 259, 97–100.

Ben-Ari, Y., Nitecka, L., Woodson, W., Robain, O., Gho, M., Cherubini, E., 1987. Vulnerabilite preferentielle des interneurones GABAergiques de l'hippocampe a l'acide kainique. L'Encephale 1, 1–10.

Bernard, C., Wheal, H., 1995. Expression of EPSP/spike potentiation following low frequency and tetanic stimulation in the CA1 area of the rat hippocampus. J. Neurosci. 15, 6542–6551.

Bernard, C., Wheal, H., 1996. A role for synaptic and network plasticity in controlling epileptiform activity in CA1 in the kainic acid-lesioned rat hippocampus in vitro. J. Physiol. (Lond.) 495, 127–142.

Bernard, C., Esclapez, M., Agid, F., Ben-Ari, Y., Hirsch, J.C., 1997. Selective loss of GABAergic inhibition in the apical dendrites of CA1 pyramidal neurons in temporal lobe epilepsy. Soc. Neurosci. Abstr. 23, 2157.

Best, N., Mitchell, J., Wheal, H.V., 1994. Ultrastructure of parvalbumin-immunoreactive neurons in the CA1 area of the rat hippocampus following a kainic acid injection. Acta Neuropathol. (Berl.) 87, 187–195.

Chagnac-Amitai, Y., Connors, B.W., 1989. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. J. Neurophysiol. 61, 747–758.

Chan, D., 1992. A topographical analysis of hippocampal field connectivity in the rat. PhD thesis, University College London.

DeLanerolle, N., Kim, J., Robbins, R., Spencer, D., 1989. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res. 495, 387–395.

Esclapez, M., Houser, C.R., 1994. Somatostatin neurons are a subpopulation of GABA neurons in the rat dentate gyrus: evidence from co-localization of pre-prosomatostatin and glutamate decarboxylase mRNAs. Neuroscience 64, 339–355.

Esclapez, M., Hirsch, J.C., Khazipov, R., Ben-Ari, Y., Bernard, C., 1997. Operative GABAergic inhibition in hippocampal CA1 pyramidal neurons in experimental epilepsy. Proc. Natl. Acad. Sci. USA 94, 12151–12156.

Franck, J., Kunkel, D., Baskin, D., Schwartzkroin, P., 1988. Inhibition in kainate-lesioned hyperexcitable hippocampi: physiological, autoradiographic and immunocytochemical observations. J. Neurosci. 8, 1991–2002.

Freund, T.F., Buzsáki, G., 1996. Interneurons of the hippocampus. Hippocampus 6, 347–470.

Gibbs, J.W., Shumate, M.D., Coulter, D.A., 1997. Differential epilepsy-assiociated alterations in postsynaptic GABAA receptor function in dentate granule cells and CA1 neurons. J. Neurophysiol. 77, 1924–1938.

Gottlieb, D., Cowan, W., 1973. Autoradiographic studies of the commissural and ipsilateral association connections of the hippocampus and the dendate gyrus. I. The commissural connections. J. Comp. Neurol. 149, 393–422.

Haas, K.Z., Sperber, E.F., Moshé, S.L., Stanton, P., 1996. Kainic acid-induced seizures enhance dentate gyrus inhibition by downregulation of $GABA_B$ receptors. J. Neurosci. 16, 4250–4260.

Hirsch, J.C., Quesada, O., Esclapez, M., Gozlan, H., Ben-Ari, Y., Bernard, C., 1996. Enhanced NMDAR-dependent epileptiform activity is controlled by oxidizing agents in a chronic model of temporal lobe epilepsy. J. Neurophysiol. 76, 4185–4189.

Houser, C.R., Esclapez, M., 1996. Vulnerability and plasticity of the GABA system in the pilocarpine model of spontaneous recurrent seizures. Epilepsy Res. 26, 207–218.

Ishizuka, N., Weber, J., Amaral, D., 1990. Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. J. Comp. Neurol. 295, 580–623.

Jefferys, J.G.R., Whittington, M.A., 1996. Review of the role of inhibitory neurons in chronic epileptic foci induced by intracerebral tetanus toxin. Epilepsy Res. 26, 59–66.

Kapur, J., Stringer, J.L., Lothman, E.W., 1989. Evidence that repetitive seizures in the hippocampus cause a lasting reduction of GABAergic inhibition. J. Neurophysiol. 61, 417–426.

Kapur, J., Macdonald, R.L., 1997. Rapid seizure-induced reduction of benzodiazepine and Zn^{2+} sensitivity of hippocampal dentate granule cell $GABA_A$ receptors. J. Neurosci. 17, 7532–7540.

Khalilov, I., Esclapez, M., Medina, I., Aggoun, D., Lamsa, K., Leinekugel, X., Khazipov, R., Ben-Ari, Y., 1997. A novel in vitro preparation: the intact hippocampal formation. Neuron 19, 743–749.

Kneisler, T.B., Dingledine, R., 1995a. Spontaneous and synaptic input from granule cells and perforant path to dentate basket cells in rat hippocampus. Hippocampus 5, 151–164.

Kneisler, T.B., Dingledine, R., 1995b. Synaptic input from CA3 pyramidal cells to dentate basket cells in rat hippocampus. J. Physiol. (Lond.) 487, 125–146.

Lanerolle, N.C., Kim, J.H., Robbins, R.J., Spencer, D.D., 1989. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res. 495, 387–395.

Laurberg, S., 1979. Commissural and intrinsic connections of the rat hippocampus. J. Comp. Neurol. 184, 685–708.

Li, X.-G., Somogyi, P., Ylinen, A., Buzsáki, G., 1994. The hippocampal CA3 network: an in vivo intracellular labeling study. J. Comp. Neurol. 339, 181–208.

Mangan, P.S., Lothman, E.W., 1996. Profound disturbances of pre- and postsynaptic $GABA_B$-receptor-mediated processes in region CA1 in a chronic model of temporal lobe epilepsy. J. Neurophysiol. 76, 1282–1296.

Marco, P., Sola, R., Alijarde, M., Sanchez, A., Ramon y Cajal, S., DeFelipe, J., 1996. Inhibitory neurons in the human epileptogenic temporal neocortex. An immunocytochemical study. Brain 119, 1327–1347.

Mathern, G.W., Babb, T.L., Pretorius, J.K., Leite, J.P., 1995. Reactive synaptogenesis and neuron densities for neuropeptide Y, somatostatin, and glutamate decarboxylase immunoreactivity in the epileptogenic human fascia dentata. J. Neurosci. 15, 3990–4004.

Morin, F., Beaulieu, C., Lacaille, J.-C., 1996. Changes in EPSCs and IPSCs of hippocampal CA1 interneurons and pyramidal cells in the kainate model of epilepsy. Soc. Neurosci. Abstr. 22, 2098.

Nadler, J., Perry, B., Cotman, C., 1978. Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. Nature 271, 676–677.

Nadler, J., Perry, B., Cotman, C., 1980a. Selective reinnervation of hippocampal area CA1 and fascia dentate after destruction of CA3-CA4 afferents with kainic acid. Brain Res. 182, 1–9.

Nadler, J., Perry, B., Gentry, C., Cotman, C., 1980b. Loss and reacquisition of hippocampal synapses after selective destruction of hippocampal synapses after selective destruction of CA3-CA4 afferents with kainic acid. Brain Res. 191, 387–403.

Nakajima, S., Franck, J., Bilkey, D., Schwartzkroin, P., 1991. Local circuit synaptic interactions between CA1 pyramidal cells and interneurons in the kainate-lesioned hyperexcitable hippocampus. Hippocampus 1, 67–78.

Obenaus, A., Esclapez, M., Houser, C.R., 1993. Loss of glutamate decarboxylase mRNA-conatining neurons in the rat dentate gyrus following pilocarpine-induced seizures. J. Neurosci. 13, 4470–4485.

Perez, Y., Morin, F., Jutras, I., Beaulieu, C., Lacaille, J.-C., 1996. CA1 pyramidal cells in hyperexcitable slices of

kainate-treated rats show sprouting of local axon collaterals. Eur. J. Neurosci. 8, 736–748.

Phelps, S., Mitchell, J., Wheal, H., 1991. Changes to synaptic ultrastructure in field CA1 of the rat hippocampus following intracerebroventricular injection of kainic acid. Neuroscience 40, 687–699.

Prince, D.A., 1978. Neurophysiology of epilepsy, Annu. Rev. Neurosci. 1, 395–415.

Prince, D.A., Jacobs, K.M., Salin, P.A., Hoffman, S., Parada, I., 1997. Chronic focal neocortical epileptogenesis: Does disinhibition play a role? Can. J. Physiol. Pharmacol. 75, 500–507.

Rempe, D.A., Bertram, E.H., Williamson, J.M., Lothman, E.W., 1997. Interneurons in area CA1 stratum radiatum and stratum oriens remain functionally connected to excitatory synaptic input in chronically epileptic animals. J. Neurophysiol. 78, 1504–1515.

Represa, A., Ben-Ari, Y., 1992. Kindling is associated with the formation of novel mossy fibre synapses in the CA3 region. Trends in Neurosci. 92, 69–78.

Represa, A., Tremblay, E., Ben-Ari, Y., 1987. Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. Neuroscience 20, 739–748.

Represa, A., Robain, O., Tremblay, E., Ben-Ari, Y., 1989. Hippocampal plasticity in childhood epilepsy. Neurosci. Lett. 99, 351–355.

Ribak, C.E., Peterson, G.M., 1991. Intragranular mossy fibers in rats and gerbils form synapses with the somata and proximal dendrites of basket cells in the dentate gyrus. Hippocampus 1, 355–364.

Ribak, C.E., Seress, L., 1983. Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. J. Neurocytol. 12, 577–597.

Robbins, R.J., Brines, J.L., Kim, J.H., Adrian, T., de Lanerolle, N., Welsh, S., Spencer, D.D., 1991. A selective loss of somatostatin in the hippocampus of patients with temporal lobe epilepsy. Ann. Neurol. 29, 325–332.

Seil, F.J., Drake-Baumann, R., Leiman, A.M., Herndon, R.M., Tiekotter, K.L., 1994. Morphological correlates of altered neuronal activity in organotypic cerebellar cultures chronically exposed to anti-GABA agents. Brain Res. 77, 123–132.

Seress, L., Ribak, C.E., 1990a. The synaptic connections of basket cell axons in the developing rat hippocampal formation. Exp. Brain Res. 81, 500–508.

Seress, L., Ribak, C.E., 1990b. Postnatal development of the light and electron microscopic features of basket cells in the hippocampal dentate gyrus of the rat. Anat. Embryol. (Berl) 181, 547–565.

Sloviter, R.S., 1987. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 235, 73–76.

Sloviter, R.S., 1991a. Feedforward and feedback inhibition of hippocampal principal cell activity evoked by perforant path stimulation: GABA-mediated mechanisms that regulate excitability in vivo. Hippocampus 1, 31–40.

Sloviter, R.S., 1991b. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the 'dormant basket cell' hypothesis and its relevance to temporal lobe epilepsy. Hippocampus 1, 41–66.

Somogyi, P., Hodgson, A., Smith, M., Nunzi, A., Gorio, A., Wu, J.-Y., 1984. Differential populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. J. Neurosci. 4, 2590–2603.

Sundstrom, L.E., Mitchell, J., Wheal, H.V., 1993. Bilateral reorganisation of mossy fibres in the rat hippocampus after a unilateral intracerebroventricular kainic acid injection. Brain Res. 609, 321–326.

Tauck, D., Nadler, J., 1985. Evidence for functional mossy fiber sprouting in hippocampal formation of kainic acid treated rats. J. Neurosci. 5, 1016–1022.

Thompson, S.M., Gahwiler, B., 1989. Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. J. Neurophysiol. 61, 501–511.

Zipp, F., Nitsch, R., Soriano, E., Frotscher, M., 1989. Enthorinal fibers form synaptic contacts on parvalbumin-reactive neurons in the rat fascia dentata. Brain Res. 495, 161–166.