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CA3-Driven Hippocampal-Entorhinal Loop Controls Rather than Sustains *In Vitro* Limbic Seizures

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Continuous application of 4-aminopyridine (4-AP, 50 μM) to combined slices of hippocampus–entorhinal cortex obtained from adult mice induces (1) interictal discharges that initiate in the CA3 area and propagate via the hippocampal regions CA1 and subiculum to the entorhinal cortex and return to the hippocampus through the dentate gyrus; and (2) ictal discharges that originate in the entorhinal cortex and propagate via the dentate gyrus to the hippocampus proper. Ictal discharges disappear over time, whereas synchronous interictal discharges continue to occur throughout the experiment. Lesioning the Schaffer collaterals abolishes interictal discharges in CA1, entorhinal cortex, and dentate gyrus and discloses entorhinal ictal discharges that propagate, via the dentate gyrus, to the CA3 subfield. Interictal discharges originating in CA3 also prevent the occurrence of ictal events generated in the entorhinal cortex during application of Mg^{2+}-free medium. In both models, ictal discharge generation recorded in the entorhinal cortex after Schaffer collateral cut is prevented by mimicking CA3 neuronal activity through rhythmic electrical stimulation (0.25–1.5 Hz) of the CA1 hippocampal output region. Our findings demonstrate that interictal discharges of hippocampal origin control the expression of ictal epileptiform activity in the entorhinal cortex. Sectioning the Schaffer collaterals may model the chronic epileptic condition in which cell damage in the CA3 subfield results in loss of CA3 control over the entorhinal cortex. Hence, we propose that the functional integrity of hippocampal output neurons may represent a critical control point in temporal lobe epileptogenesis.

Key words: hippocampus; entorhinal cortex; seizures; CA3; 4-aminopyridine; Mg^{2+}-free

It is believed that seizures originating in the entorhinal cortex propagate to the hippocampus proper and reenter the entorhinal cortex in a loop that functions in a "loop-gain" manner to sustain and reinforce long-lasting epileptiform activity (Paré et al., 1992; Jones, 1993). The reciprocal anatomical connectivity between entorhinal cortex and hippocampus (Amaral and Witter, 1989) in addition to the cellular electrophysiological properties of both CA3 and entorhinal neurons (Schwartzkroin and Prince, 1978; Traub and Wong, 1982; Wong and Traub, 1983; Jones and Heinemann, 1988; Heinemann et al., 1993) may favor such a reinforcing mechanism, thus allowing seizure amplification.

We and others have demonstrated in rat combined hippocampus–entorhinal cortex slices that prolonged epileptiform discharges initiate in the entorhinal cortex and propagate to the hippocampal formation (Jones and Lambert, 1990; Drier and Heinemann, 1991; Avoli et al., 1992; Nagao et al., 1996). However, these electrographic seizures did not reenter the entorhinal cortex, suggesting that combined rat slices, which have preserved connections between entorhinal cortex and hippocampus, may lack functional hippocampal inputs to the entorhinal cortex. Findings obtained in the isolated guinea pig brain (Paré et al., 1992) have indicated that the hippocampal–entorhinal loop is operative in this preparation and may be involved in sustaining and amplifying limbic seizures.

In the present study, we have used combined hippocampal–entorhinal cortex slices obtained from adult mouse to investigate the role of the hippocampal–entorhinal loop in two different *in vitro* models of epileptiform discharge. Owing to the reduced size of the animal brain, we assumed that reciprocal connectivity between the entorhinal cortex and the hippocampus may be preserved. Our findings indicate that this was indeed the case. In particular, we addressed the following questions: (1) Does the hippocampal-entorhinal loop subserve a sustaining purpose? (2) What is the interaction between ictal and interictal epileptiform activity in the initiation, propagation, and control of seizures that may lead to an epileptic condition? Herein, we introduce a novel, surprising role that the hippocampal–entorhinal loop may play in limbic seizures and thus in temporal lobe epilepsy. We demonstrate that when interictal epileptiform activity of CA3 origin is allowed to propagate within this loop, rather than contributing to intensify seizure activity, reentry serves to control and thus to prevent seizure generation. On the contrary, when the loop is discontinued, seizure activity is allowed to be generated in the entorhinal cortex and to propagate to the hippocampus proper via the dentate gyrus.

MATERIALS AND METHODS

Adult, male, CD-1 or BALB/c mice (25–35 gm) were decapitated under halothane anesthesia. Their brains were quickly removed and were placed in cold (1–4°C), oxygenated artificial CSF (ACSF). Horizontal slices (550 μm thick) were cut with a vibratome and then transferred to a tissue chamber where they lay between oxygenated ACSF and humidified gas (95%O_2, 5%CO_2) at 32–33°C. ACSF composition was (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10. 4-AP (50 μM) was bath-applied. In some experiments, epi-

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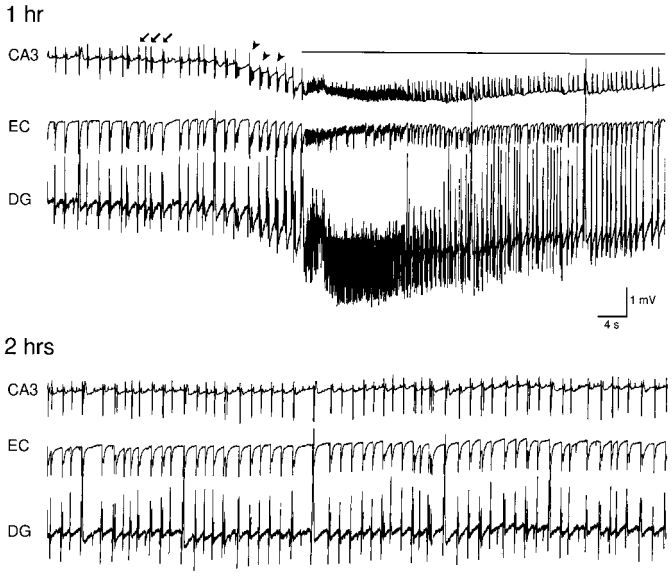

Figure 1. Spontaneous epileptiform activity recorded at 1 and 2 hr during continuous bath application of 4-AP. Simultaneous field potential recordings were made in the CA3 stratum radiatum, the deep layers of the entorhinal cortex, and the dentate granule cell layer. Ictal discharge, recorded at 1 hr, is indicated by *continuous line*, interictal discharges by *arrows*, and robust interictal discharges with afterdischarge component by *arrowheads*. At 2 hr during 4-AP application, ictal discharges disappear. In this and the following figures, *EC* and *DG* stand for entorhinal cortex and dentate gyrus, respectively.

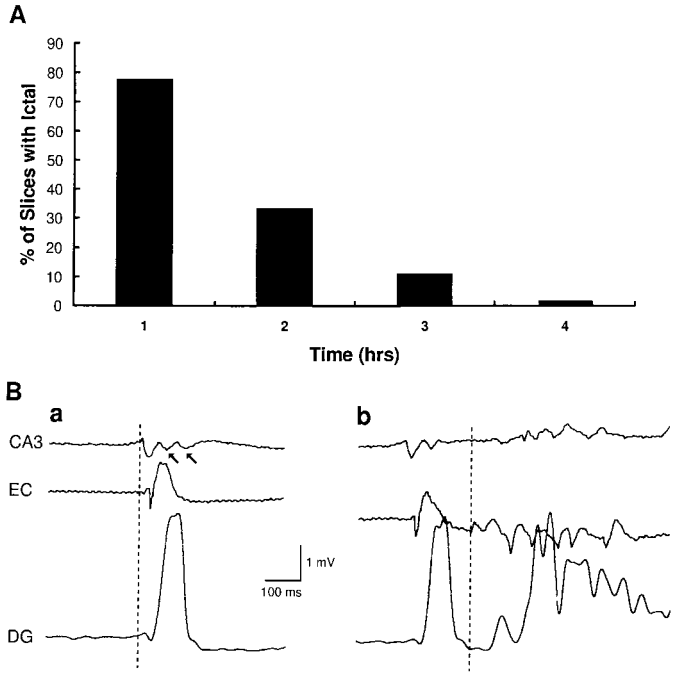

Figure 2. *A*, Percentage histogram of slices generating ictal discharges at different times of 4-AP application. These slices ($n = 9$) were recorded for >4 hr. *B*, Expanded traces of interictal (*a*) and ictal discharges (*b*) induced by 4-AP (~1 hr) in an intact entorhinal-hippocampal combined slice. In *a*, the interictal discharge initiates in the CA3 region and propagates to the entorhinal cortex and the dentate gyrus; *arrows* point at the late components of the interictal discharge recorded in CA3. In *b*, the ictal discharge is preceded by an interictal event with temporal profile similar to that seen in *a*, whereas the site of origin of the ictal discharge appears to occur in the entorhinal cortex. *Dotted lines* in *a* and *b* were positioned at the time of the earliest visible deflection in the three field potential recordings.

leptiform discharges were induced by Mg^{2+}-free ACSF. Chemicals were acquired from Sigma (St. Louis, MO).

Slices included the entorhinal cortex and the hippocampus proper that also comprised the subiculum and the dentate gyrus. Field potential recordings were performed with ACSF-filled microelectrodes (tip diameter, ≤8 μm; resistance, 2–10 MΩ) that were positioned in the medial portion of the entorhinal cortex, the granule cell layer of the dentate gyrus, and the CA3 or CA1 stratum radiatum. Signals were fed to high-impedance amplifiers, displayed on a Gould-pen chart recorder, and also digitized and stored on videotape for subsequent analysis.

A series of cutting experiments was performed using a microknife to establish the origin and the pathway used by the epileptiform activity to propagate in the slice. Time delay measurements for initiation of epileptiform discharges were performed by taking as a temporal reference the first deflection from the baseline recording. Extracellular stimulation was performed with a bipolar stainless steel electrode that was placed in the stratum radiatum of the CA1 subfield or in the deep layers of the entorhinal cortex. Stimulation parameters were intensity, 0.1–0.5mA; duration, 100 μsec; and frequency, 0.5–1.5 Hz. The stimulation intensity was adjusted to obtain an interictal discharge. Measurements in the text are expressed as mean ± SD, and *n* represents the number of slices studied. Data were compared with the Student's *t* test or the ANOVA test and were considered significantly different if $p < 0.05$.

RESULTS

Properties of 4-AP-induced epileptiform discharges

Simultaneous field potential recordings in CA3, dentate gyrus, and entorhinal cortex were performed in >70 combined hippocampus–entorhinal cortex slices. Most recordings in the entorhinal cortex were done in the deep layers (~500–800 μm from the pia), where maximal field potential amplitudes were detected (cf. Avoli et al., 1992). Bath application of 4-AP induced the appearance of spontaneous activity that matured within 1 hr to give rise to two main types of activity that occurred synchronously in all areas. The first type of epileptiform activity consisted of brief interictal-like (thereafter termed interictal) events that lasted 130–350 msec in CA3, 200–450 msec in the entorhinal cortex, and 120–250 msec in the dentate gyrus and occurred at intervals of 1.3 ± 0.3 sec (Fig. 1, *arrows*; 1 hr). The second type of synchronous discharge consisted of prolonged, ictal-like (thereafter called ictal) discharges that lasted 15–78 sec in all areas and occurred at intervals of 3.9 ± 0.9 min (Fig. 1, *continuous line*; 1 hr). Interictal discharges with prominent afterdischarge (up to 2 sec) often occurred before the onset of an ictal event (Fig. 1, *arrowheads*; 1 hr).

The ictal epileptiform activity induced by 4-AP changed over time. As illustrated in Figure 1 (2 hr), ictal discharges disappeared and were replaced by continuous interictal events, which remained synchronous in the entorhinal and hippocampal regions. In nine slices, the activity was monitored from the start of 4-AP application throughout a period of 4 hr. The percentage of slices generating ictal discharges at different times after the onset of 4-AP application is shown in Figure 2*A*.

By contrast, interictal discharges did not show over time any detectable change at the field potential level. In six slices we investigated the site of origin and the modalities of propagation of the interictal discharges in the hippocampal–entorhinal network by performing time delay measurements. This type of analysis showed that interictal events initiated in CA3, propagated (presumably via the CA1 and the subiculum) to the entorhinal cortex, and returned to the hippocampus through the dentate gyrus to give rise to a second or third interictal component in CA3 both when ictal discharges were still present (Fig. 2*B*, *arrows*) and

Table 1. Time delays for interictal discharges

CA3 → EC (msec)	EC → DG (msec)	DG → CA3 (msec)
28.0 ± 12.1	22.5 ± 12.8	35.0 ± 23.8
$n = 6$	$n = 6$	$n = 6$

when ictal activity had disappeared (see Fig. 4*B*, before SC cut). The time delays between the interictal discharges in the different areas are illustrated in Table 1.

Changes induced by neuronal pathway sections

It was difficult from time delay measurements to determine the site of origin of the ictal discharges. Therefore, we established the origin and pattern of propagation of the synchronous activities induced by 4-AP by cutting selective neuronal pathways. Severing the perforant path abolished the occurrence of ictal discharges in the hippocampus proper without affecting the interictal activity of CA3 origin or modifying the ictal discharges in the entorhinal cortex ($n = 3$; Fig. 3*A*).

To further establish the functional role of interictal discharge reentry to CA3 subfield (as indicated by the delay measurements) and its effect on entorhinal epileptiform activity, we sectioned the Schaffer collaterals. We reasoned that a cut of the Schaffer collaterals would selectively prevent propagation of CA3 hippocampal activity to the hippocampal efferent regions CA1 and subiculum, thus preventing hippocampal activity from reaching the entorhinal cortex. In slices that displayed ictal discharges, this section resulted in (1) blockade of interictal discharges in all but the CA3 subfield and (2) increase in the duration of ictal events from 36 ± 20.1 sec under control conditions to 69 ± 35.1 sec after Schaffer collateral cut ($n = 6$; Fig. 3*B*).

Moreover, in slices in which ictal discharges had stopped to occur over time, the Schaffer collateral section made (1) interictal events disappear in nonCA3 subfields and (2) ictal events reappear. These ictal discharges lasted 30–160 sec, occurred at intervals of 1.3–12.5 min, and were synchronous in entorhinal cortex, dentate, and CA3 areas ($n = 34$; Fig. 4*A*). In our preparation, interictal discharges recorded in CA3 consisted of multiple components (number of components in intact slice = 2.7 ± 0.7; $n = 6$) when the hippocampal–entorhinal circuit was intact (Fig. 4*B,C*). After sectioning the Schaffer collaterals, interictal discharges in entorhinal cortex and dentate gyrus ceased to occur although the number of interictal discharge components in CA3 was reduced to 1.1 ± 0.4 ($n = 6$; Fig. 4*B,C*). In very few slices, interictal discharges of entorhinal cortex origin reminiscent of those induced by pilocarpine (Nagao et al., 1996) were recorded (see Fig. 7*A*). As shown in Figure 4*A*, a further lesion of the perforant path abolished ictal discharge in both dentate gyrus and CA3, in which only interictal discharges continued to recur (Fig. 4; $n = 6$).

Hippocampal control of low-Mg^{2+}-induced epileptiform discharges

To ensure that the results obtained with 4-AP could also be reproduced with another *in vitro* type of epileptiform discharge, we repeated these experiments during application of Mg^{2+}-free ACSF ($n = 5$). This procedure is known to induce synchronized interictal discharges in the isolated hippocampal slice (Tancredi et al., 1990) and both interictal and ictal epileptiform discharges in combined entorhinal–hippocampal slices (Walther et al., 1986; Jones and Lambert, 1990a,b; Drier and Heinemann, 1991).

Spontaneous, synchronous events matured over time (~2 hr) to become robust, interictal discharges that lasted 1.3 ± 0.8 sec, occurred at 0.3 ± 0.1 Hz ($n = 5$), and appeared in all areas of the combined slice (Fig. 5, *top*). These interictal discharges initiated in the hippocampus proper (most often CA3), propagated to the entorhinal cortex, and returned to the hippocampus via the dentate gyrus (Fig. 5, *inset, top*). In the unlesioned slice, interictal discharges in CA3, entorhinal cortex, and dentate gyrus consisted of multiple components (Fig. 5, *inset, bottom*). Cutting the Schaffer collaterals reduced the number of interictal discharge components in CA3 and dentate gyrus to one and abolished interictal activity in the entorhinal cortex (Fig. 5, *bottom*). In addition, this procedure disclosed ictal discharges that occurred in apparent synchronous manner in all areas (Fig. 5, *bottom*); they lasted 19.6 ± 9.5 sec and repeated at an interval of 49.1 ± 25.4 sec ($n = 5$). Sectioning the perforant path ($n = 2$) abolished seizure propagation to the dentate gyrus and CA3 subfield, further demonstrating that ictal discharges originate in the entorhinal cortex (not illustrated).

Blockade of ictal discharges by low frequency stimulation

To further demonstrate that the interictal events originating in CA3 function in a network to prevent ictal activity from being

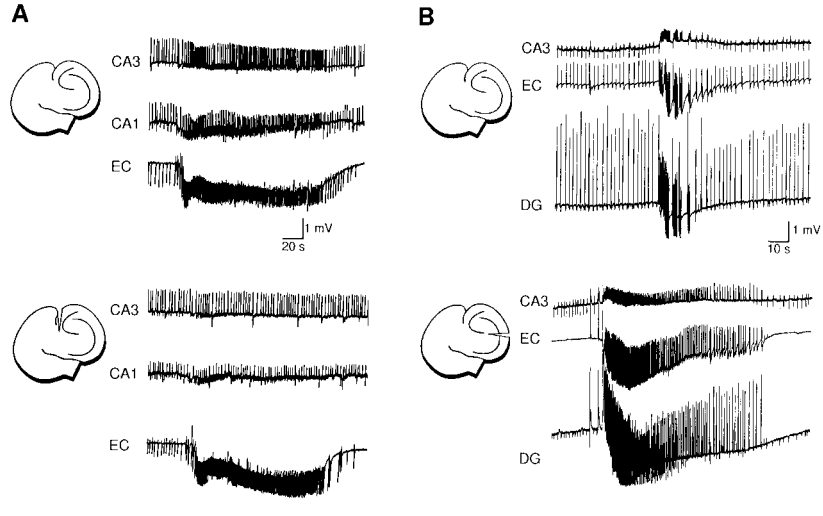

Figure 3. Spontaneous epileptiform activity recorded during application of 4-AP (~1 hr) in a combined hippocampal–entorhinal cortex slice before and after selective neuronal pathway sectioning. *A*, Changes in 4-AP-induced activity before and after cut of the perforant path indicate that the ictal discharges originate in the entorhinal cortex. *B*, Effects induced by Schaffer collateral cut further demonstrate that interictal discharges initiate in the CA3 subfield, because they are abolished in the entorhinal cortex and dentate gyrus. Note also that after Schaffer collateral cut, the ictal discharge duration is increased ($n = 6$; $p < 0.05$).

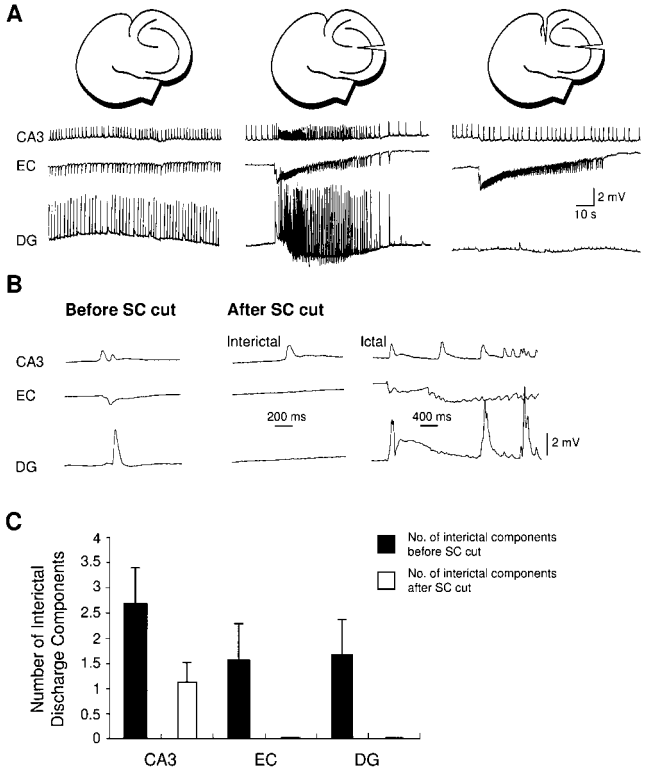

Figure 4. Effects induced by sectioning the Schaffer collaterals and the perforant pathway on epileptiform discharges recorded ~2 hr after continuous application of 4-AP. *A*, Interictal discharges are recorded in CA3, entorhinal cortex, and dentate gyrus in the intact slice (*traces, left*). Sectioning the Schaffer collaterals abolishes the interictal discharges in entorhinal cortex and dentate gyrus and discloses an ictal discharge that is simultaneously recorded in CA3, entorhinal cortex, and dentate gyrus (*traces, middle*). Further cutting of the perforant path abolishes the propagation of the ictal discharge to the entorhinal cortex and dentate gyrus (*traces, right*). *B*, Expanded traces from the experiment shown in *A* demonstrate that interictal discharges reenter CA3. They comprise two components in the CA3 of the intact slice (*Before SC cut*), whereas after sectioning the Schaffer collaterals, a single component is left (*After SC cut*, *Interictal*). The onset of an ictal discharge recorded after Schaffer collateral cut is also shown (*After SC cut*, *Ictal*). *C*, Quantitative summary of the effects induced by Schaffer collateral cut on the number of interictal discharge components in CA3, entorhinal cortex (*EC*) and dentate gyrus (*DG*) ($n = 6$; $p < 0.05$).

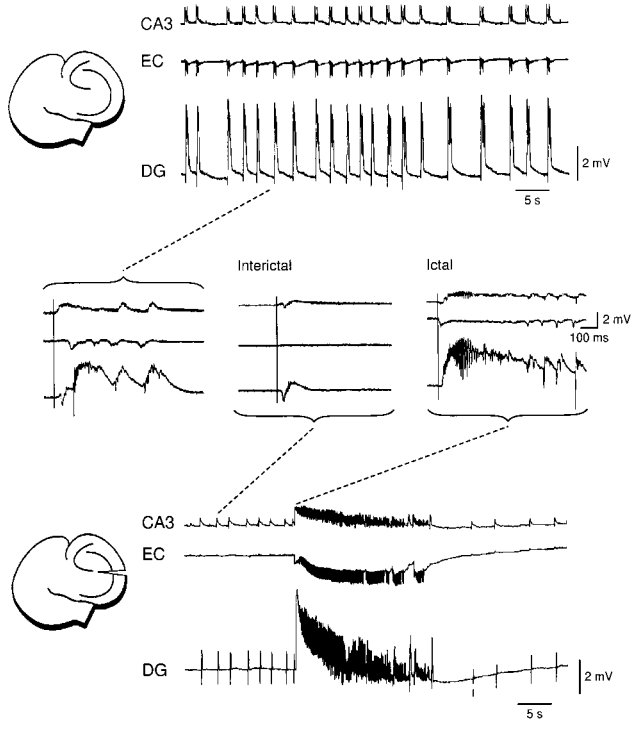

Figure 5. Spontaneous epileptiform activity induced by Mg^{+2}-free ACSF before and after Schaffer collateral cut. Before the lesion (*top*), synchronized interictal discharges are recorded in CA3, entorhinal cortex, and dentate gyrus. Sectioning the Schaffer collaterals (*bottom*) abolishes interictal discharges in the entorhinal cortex and discloses ictal epileptiform activity that is recorded in the three areas. Expanded traces of the experiment shown in the *top* and *bottom* are illustrated in the *middle*. Note that before the Schaffer collateral cut Mg^{+2}-free-induced interictal discharges consist of multiple components, whereas after the cut (*Interictal*) they are markedly reduced in duration and number of events. Note also that the ictal discharge (*Ictal*) is initiated in the entorhinal cortex.

generated in the entorhinal cortex, we stimulated the stratum radiatum of the hippocampal output region CA1 (Amaral and Witter, 1989; Tamamaki and Nojyo, 1995) at 0.25–1.5 Hz (i.e., the interictal frequency observed before lesioning the Schaffer collateral pathway). This procedure abolished the ictal activity induced either by application of Mg^{2+}-free ACSF ($n = 2$; Fig. 6*B*) or by 4-AP ($n = 9$; Fig. 7*A,B*) for the duration of the stimulation period. When the stimulation was interrupted, ictal episodes reappeared at the same frequency as before stimulation. This effect could be reproduced by a successive stimulation protocol within the same experiment (Fig. 7*B*). In three slices, low-frequency stimulation was performed for an extended period (20 min) during which ictal events never occurred.

DISCUSSION

In this study we used combined mouse hippocampal–entorhinal slices in which reciprocal connections between hippocampus and entorhinal cortex are preserved to investigate the interaction of these structures in two *in vitro* models of limbic seizures. Clinical neurophysiological investigations have shown that functional connections between hippocampus and entorhinal cortex exist in patients with temporal lobe epilepsy (Ruteki et al., 1989; Spencer and Spencer, 1994). The main findings reported here can be summarized as follows. First, 4-AP- and Mg^{2+}-free-induced epileptiform interictal activity originating in the hippocampus reenters this region after propagating through the hippocampal–entorhinal loop. Second, the function of this network loop is to prevent the generation of prolonged, ictal epileptiform activity that initiates in the entorhinal cortex. Third, low-frequency stimulation of hippocampal output regions (a procedure that mimics CA3 interictal activity) prevents the generation of ictal discharges in the entorhinal cortex. Because the Schaffer collateral cut may mimic the selective hippocampal neuronal loss seen in temporal lobe epilepsy patients as well as in chronic models of epileptiform discharge (Ben-Ari, 1985; Turski et al., 1989; Gloor, 1991), our findings suggest that a decreased function of hippocampal outputs with no structural rearrangement of hippocampal circuits may be sufficient to facilitate the occurrence of intermittent spontaneous seizures reminiscent of the chronic epileptic condition.

Reentry within the entorhinal–hippocampal loop

In the intact, normal brain, sharp waves originating in the hippocampus (most probably in CA3) propagate to the deep layers of the entorhinal cortex and then to other brain regions but not to

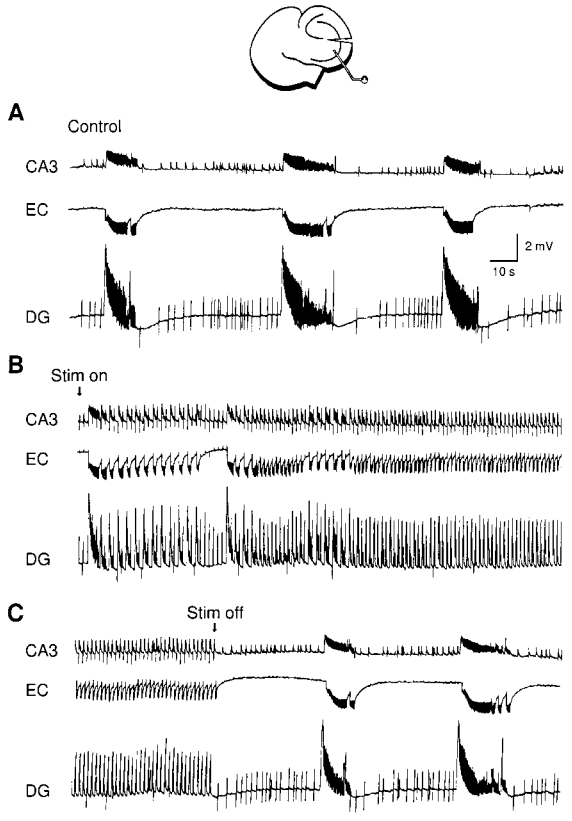

Figure 6. Effect induced by extracellular stimuli delivered in the CA1 subfield (1 Hz) on the Mg^{+2}-free-induced ictal activity recorded after Schaffer collateral cut. *A–C*, Continuous recordings demonstrate that low-frequency stimulation prevents the occurrence of ictal discharges that reappear on termination of the stimulation.

the superficial layers of the entorhinal cortex, and thus they do not reenter the hippocampus (Chrobak and Buzsáki, 1994). Indeed, reentry may only occur during abnormal epileptiform activity, such as described herein. Reverberation of activity between the entorhinal and hippocampal structures has been shown to sustain and amplify prolonged epileptiform events (Paré et al., 1992). Ictal discharges originate in the entorhinal cortex (Jones and Lambert, 1990; Drier and Heinemann, 1991; Avoli et al., 1992; Nagao et al., 1996), whereas interictal activity is mainly generated in area CA3 of the hippocampus (Schwartzkroin and Prince, 1978; Wong and Traub, 1983; Voskuyl and Abus, 1985; Perrault and Avoli, 1991). Therefore the epileptogenic potential of both structures may support the view that reentry of activity within the hippocampal–entorhinal loop sustains and amplifies prolonged epileptiform activity through re-excitation (Paré et al., 1992).

In the present study, spontaneous potentials induced by either 4-AP or Mg^{+2}-free medium were recorded synchronously in all areas of the combined slice, suggesting that reciprocal connections between the entorhinal cortex and the hippocampus proper do exist in our preparation and, thus, that epileptiform discharges do propagate within the hippocampal–entorhinal loop. Lesioning the Schaffer collaterals influenced the epileptiform activity recorded in the entorhinal cortex in two ways. First, it blocked the occurrence of interictal discharges originating in the hippocampus proper. In particular, we showed that the CA3 interictal discharges recorded in the intact slice consist of multiple components in both the 4AP and Mg^{2+}-free models. Schaffer collateral cut during 4-AP application abolished interictal discharge components in the entorhinal cortex and the dentate gyrus and reduced them to unity in CA3. This procedure also blocked the occurrence of Mg^{2+}-free-induced interictal discharge components in the entorhinal cortex and reduced the number to unity in both CA3 and dentate areas. Second, Schaffer collateral lesion disclosed the appearance of robust ictal discharges in both 4AP and Mg^{+2}-free conditions. Ictal discharges induced by 4AP or Mg^{+2}-free ACSF in rat combined slices originate in the entorhinal cortex (Jones and Heinemann, 1988; Avoli et al., 1992). This was confirmed here for both 4-AP- and Mg^{+2}-free-induced ictal events, because perforant path cut abolished ictal activity in the hippocampus but not in the entorhinal cortex. The complexity of the ictal discharge waveform did not allow us to identify ictal discharge reentry clearly in the entorhinal cortex. Nonetheless, 4-AP-induced ictal discharges, when present in an unlesioned slice, were prolonged after cut of the hippocampal outputs. In addition, when only interictal discharges were recorded in the intact slice treated with 4AP or Mg^{+2}-free medium, Schaffer collateral lesion disclosed ictal events.

Taken together, these data demonstrate that interictal activity requires an intact hippocampal–entorhinal loop to propagate to all areas of the combined slice and to reenter the hippocampus after having propagated to the entorhinal cortex. Moreover, these findings reveal that if ictal activity does reenter the hippocampal–entorhinal loop, the result is likely to have no reinforcing effect. In fact, ictal discharges, in contrast to interictal activity, are favored by a discontinued loop, in which hippocampal output activity does not reach the entorhinal cortex. Thus our work demonstrates that contrary to the common view, amplification of epileptiform activity through the hippocampal–entorhinal loop occurs in the hippocampus (most probably in CA3) for the interictal, not in the entorhinal cortex for the ictal discharge, and that the role of reentrant activity is to prevent rather than to sustain prolonged ictal events.

Interictal–ictal interaction

The presence of a preserved hippocampal–entorhinal synaptic loop through which epileptiform activity can propagate has allowed us to determine the interaction between ictal and interictal discharges, each of which is generated at distinct sites within the circuit. Some reports have proposed that interictal activity contributes to the transition from interictal to ictal discharge (Prince et al., 1983; Jensen and Yaari, 1988), yet other studies have showed that interictal activity interferes with ictal discharge generation (Swartzwelder et al., 1987; Bragdon et al., 1992). As demonstrated in an earlier report (Avoli et al., 1992), here we confirm that when ictal discharges are abolished in the hippocampus through perforant path cut, interictal activity is unaffected. Our results also show that Schaffer collateral cut, which abolishes interictal discharges in the entorhinal cortex, discloses ictal discharges in that region. Moreover, when interictal discharges are blocked in the entorhinal cortex and ictal events are disclosed, low-frequency stimulation of hippocampal output CA1 area inhibits the occurrence of ictal discharges. Hence, these findings demonstrate the blocking effect that interictal discharges can exert on the generation of ictal activity in the entorhinal cortex.

Acute models with chronic properties

Human temporal lobe epilepsy is associated with hippocampal sclerosis in which dentate hilus, CA3, and CA1 neurons are lost (Gloor, 1991; Wieser et al., 1993) and is accompanied by mossy

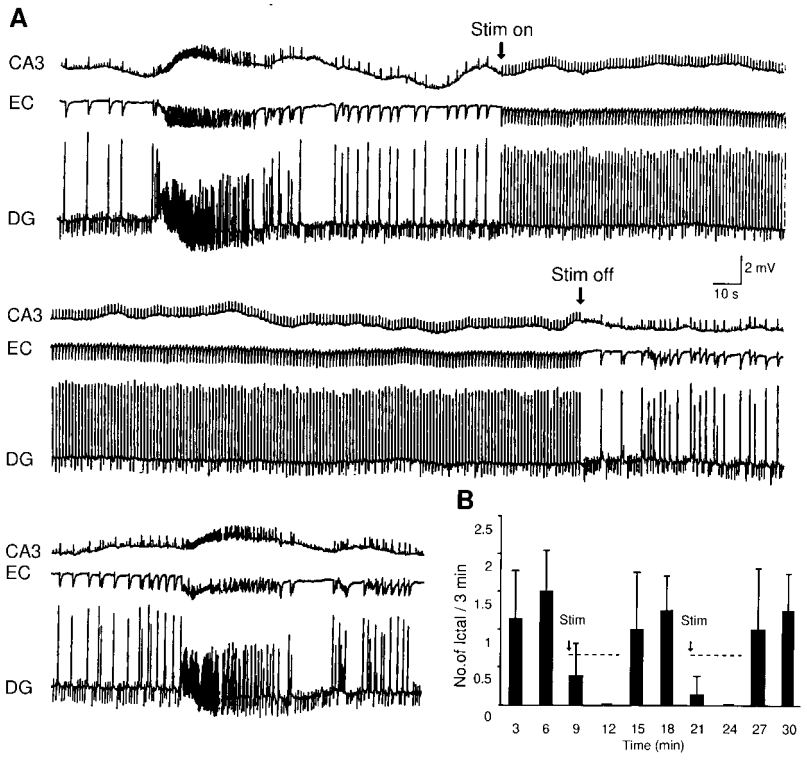

Figure 7. *A*, Continuous recordings showing the effect of CA1 stimulation at 1 Hz on the 4-AP-induced epileptiform activity recorded after Schaffer collateral cut. Note the persistence of interictal discharges, presumably of entorhinal origin, before and after the ictal activity. Ictal discharge does not occur during the stimulation period and re-appear on termination of the stimulation. *B*, Time histogram showing the effect of low-frequency stimulation on ictal discharge occurrence ($p < 0.05$ for both stimulation protocols). Data were obtained from eight slices for the first stimulation and four slices for the second stimulation protocol.

fiber sprouting (Sutula et al., 1989; Represa et al., 1989a; Houser et al., 1990). Similar neuropathological findings have been reported in *in vivo* experimental models of epilepsy such as kainic acid-lesioned rat hippocampus (Ben-Ari, 1985) or pilocarpine-treated rats (Turski et al., 1989), in which CA3 neurons and their synapses onto CA1 pyramidal cells are susceptible to cell death. Mossy fiber sprouting has also been demonstrated in chronically epileptic animals (Sutula et al., 1988; Represa et al., 1989b; Cavazos et al., 1991). Whether cell loss, synaptic reorganization, or a combination of these factors causes the epileptic condition remains controversial. However, recent findings suggest that pilocarpine-treated animals in which mossy fiber sprouting was blocked by injection of a protein synthesis inhibitor still present recurrent seizures (Longo and Mello, 1996).

The acute *in vitro* mouse model described here may address directly the question of whether cell loss alone can be a cause of chronic epilepsy. Sectioning the Schaffer collaterals in our slice preparation may selectively mimic the cell damage and synapse loss observed in animal models of temporal lobe epilepsy (without any other plastic changes). Therefore, our findings reveal that functional CA3 neurons and hippocampal outputs are critical in controlling the chronic state of spontaneous recurrent seizures. Nagao et al. (1994) have demonstrated that 4-AP-induced CA3 interictal discharges occur at a reduced frequency in a hippocampal slice obtained from a rat with long-term pilocarpine seizures compared with age-matched controls. Epileptiform activity induced in the dentate gyrus is lengthened in kindled animals (Stringer and Lothman, 1989). Moreover, loss of entorhinal layer III neurons (thought to alter the communication between entorhinal layers and thus to interfere with the hippocampal–entorhinal loop) is observed in chronic epileptic animals (Du et al., 1995). Noting that CA3 neurons are lost in pilocarpine-treated animals (Turski et al., 1989; Liu et al., 1994), it is attractive to speculate that a decreased ability for interictal discharge generation in CA3 may perturb the control of chronically recurrent seizures.

Ictal activity in the entorhinal cortex is elaborated and sustained by an intrinsic excitatory circuit within this structure (Ijima et al., 1996). Thus, CA3-driven rhythmic inputs such as brief interictal discharges may perturb the ability of the entorhinal circuit to reverberate and thus to express ictal activity. Therefore, cell and/or synapse loss between the CA3 and CA1 regions of the hippocampus may be sufficient to prevent the hippocampal-driven control of spontaneous recurrent seizures of entorhinal origin. This view is in line with the ability of low-frequency stimulation of surviving CA1 afferents (1 Hz for 15 min) to reduce epileptiform activity in the kainic acid lesioned rat hippocampus (Bernard and Wheal, 1996). Hence, low frequency (0.25–1.5 Hz) stimulation of the hippocampal outputs in patients with temporal lobe epilepsy may represent the basis for a new direction in clinical epilepsy research.

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