Chronic Changes in Synaptic Responses of Entorhinal and Hippocampal Neurons After Amino-Oxyacetic Acid (AOAA)–Induced Entorhinal Cortical Neuron Loss

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[1]*Neurology Research Center, Helen Hayes Hospital, West Haverstraw, New York 10993-1195;* [2]*Departments of Pharmacology and Neurology, Columbia University, College of Physicians and Surgeons, New York City, New York 10032; and* [3]*Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, Maryland 21228*

Scharfman, H. E., J. H. Goodman, F. Du, and R. Schwarcz. Chronic changes in synaptic responses of entorhinal and hippocampal neurons after amino-oxyacetic acid (AOAA)–induced entorhinal neuron loss. *J. Neurophysiol.* 80: 3031–3046, 1998. Synaptic responses of entorhinal cortical and hippocampal neurons were examined in vivo and in vitro, 1 mo to 1.5 yr after a unilateral entorhinal lesion caused by a focal injection of amino-oxyacetic acid (AOAA). It has been shown previously that injection of AOAA into the medial entorhinal cortex produces cell loss in layer III preferentially. Although behavioral seizures stopped ∼2 h after AOAA treatment, abnormal evoked responses were recorded as long as 1.5 yr later in the entorhinal cortex and hippocampus. In the majority of slices from AOAA-treated rats, responses recorded in the superficial layers of the medial entorhinal cortex to white matter, presubiculum, or parasubiculum stimulation were abnormal. Extracellularly recorded responses to white matter stimulation were prolonged and repetitive in the superficial layers. Intracellular recordings showed that residual principal cells in superficial layers produced prolonged, repetitive excitatory postsynaptic potentials (EPSPs) and discharges in response to white matter stimulation compared with brief EPSPs and a single discharge in controls. Responses of deep layer neurons of AOAA-treated rats did not differ from controls in their initial synaptic response. However, in a some of these neurons, additional periods of excitatory activity occurred after a delay. Abnormal responses were recorded from slices ipsilateral as well as contralateral to the lesioned hemisphere. Recordings from the entorhinal cortex in vivo were abnormal also, as demonstrated by prolonged and repetitive responses to stimulation of the area CA1/subiculum border. Evoked responses of hippocampal neurons, recorded in vitro or in vivo, demonstrated abnormalities in selected pathways, such as responses of CA3 neurons to hilar stimulation in vitro. There was a deficit in the duration of potentiation of CA1 population spikes in response to repetitive CA3 stimulation in AOAA-treated rats. Theta activity was reduced in amplitude in area CA1 and the dentate gyrus of AOAA-treated rats, although evoked responses to angular bundle stimulation could not be distinguished from controls. The results demonstrate that a preferential lesion of layer III of the entorhinal cortex produces a long-lasting change in evoked and spontaneous activity in parts of the entorhinal cortex and hippocampus. Given the similarity of the lesion produced by AOAA and entorhinal lesions in temporal lobe epileptics, these data support the hypothesis that preferential damage to the entorhinal cortex contributes to long-lasting changes in excitability, which could be relevant to the etiology of temporal lobe epilepsy.

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INTRODUCTION

The neuropathology of patients with temporal lobe epilepsy varies greatly (Babb and Brown 1987; Bruton 1988; Falconer et al. 1964; Kim et al. 1990; Meldrum and Bruton 1992). One of the most common patterns of damage involves the hippocampus, where cell loss often is observed in the pyramidal cell layers (Sommer's sector) (Sommer 1880) and hilus (endfolium) (Margerison and Corsellis 1966). However, cell loss also has been reported in other areas of the brain, such as the parahippocampal region, including the entorhinal cortex (Gastaut et al. 1959; Levesque et al. 1991; Morin and Gastaut 1954). In fact, cases have been reported where damage to the entorhinal cortex is evident without detectable hippocampal pathology (Gastaut et al. 1959). These cases raise the question whether damage to the entorhinal cortex is sufficient to cause hyperexcitability in the temporal lobe (Morin and Gastaut 1954; Rutecki et al. 1989; Spencer and Spencer 1994). An alternative perspective is that damage in the entorhinal cortex could be merely a result of seizure activity or a byproduct of another, more fundamental abnormality elsewhere.

An animal model of epilepsy with pathology in the entorhinal cortex could be used to answer this question. Such a model was developed by Du and Schwarcz (1992), who injected amino-oxyacetic acid (AOAA) into the entorhinal cortex of anesthetized adult rats and found that layer III neurons were damaged preferentially. Silver degeneration studies in these animals show that neurons also degenerate at the CA1/subiculum border and, less consistently, in the temporal cortex, perirhinal cortex, and lateral amygdala (Du et al. 1998). Importantly, AOAA-treated rats display behavioral seizures lasting ∼2 h immediately after recovery from anesthesia. After these seizures end, animal behavior appears normal.

Although chronic, spontaneous behavioral seizures have so far not been observed after an intraentorhinal AOAA injection, there still could be changes in excitability. This study was undertaken to address whether this might be the case, and hence, whether a preferential lesion of the superficial layers

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of the entorhinal cortex produces any long-lasting changes in excitability in the entorhinal cortex or hippocampus. To this end, spontaneous and evoked responses of entorhinal and hippocampal neurons in AOAA-injected rats were studied in vivo and in vitro. These recordings were compared with those from vehicle-injected, age-matched controls, and naive controls.

Some of these results have been published in abstract form (Scharfman et al. 1997).

METHODS

All procedures involving laboratory animals met the guidelines of the National Institutes of Health and the New York State Department of Health. Unless stated otherwise, all chemicals were obtained from Sigma.

Preparation of AOAA-treated rats and vehicle controls

AOAA was injected as previously described (Du and Schwarcz 1992). Briefly, adult male Sprague-Dawley rats (200–275 g; Charles River) were anesthetized with chloral hydrate (350–370 mg/kg) and placed in a stereotaxic frame. A small hole was drilled over the left entorhinal cortex at the following coordinates: 5.0 mm lateral to the midline, −2.1 mm posterior to the intersection of lambda and the midline (incisor bar, −3.3). A 1 μl Hamilton syringe filled with 0.75 μl AOAA (10 mM dissolved in 0.1 M phosphate buffer, pH 7.4) was angled at 20° to the vertical plane and inserted in the cortex. A volume of 0.25 μl was injected at three depths relative to the skull surface (in mm): −4.8, −5.3, and −5.8. After injection, the needle was left in place for 10 min and then slowly removed. Animals then were placed under a heating lamp until they had recovered from anesthesia (~30–60 min).

Of note is that these animals had behavioral seizures intermittently for ~2 h after they recovered from anesthesia. Wet-dog shakes were observed frequently, either in isolation or immediately after behavioral seizures. Staring episodes in a ''frozen'' position were common. Behavioral seizures usually reached stages 2 and 3, and occasionally stages 4 and 5 (Racine 1972). Within the 2-h period, two to three such seizures usually occurred. Behavioral seizures were never observed thereafter, although it is possible that they were missed and that seizures without a motor component occurred. Continuous electroencephalographic (EEG) monitoring for this activity was not conducted.

Nissl staining or silver degeneration stain has shown that AOAA-treated animals sustain neuronal damage mostly in layer III of the medial entorhinal cortex (Du and Schwarcz 1992; Du et al. 1998). In the present study, Nissl stain was used to confirm cell loss (see *Anatomic methods*). The results showed that the animals used in this study had lesions that were qualitatively similar to those described previously (Fig. 1), although the extent of cell loss appeared to be smaller than in other studies (Du and Schwarcz 1992; Du et al. 1998). However, although the lesion in layer III was less complete, there was some neuronal loss at the CA1/subiculum border (cf. Du et al. 1998).

In vitro studies

PREPARATION OF SLICES. Slices were made from AOAA-treated rats and vehicle-injected controls. One group was examined ~1.5 yr after AOAA or vehicle treatment. The other group was examined between 1 and 6 mo after treatment. Rats were anesthetized with ether, the brain was quickly removed, and placed in ice-cold sucrose solution (which contained, in mM, 125 sucrose, 5 KCl, 2.0 $CaCl_2$, 2.0 $MgSO_4$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 D-glucose). Slices (400-μm thick) were cut horizontally using a Vibroslice (Campden Instruments), and all slices were immediately placed in a recording chamber (modified from Fine Science Tools) where they lay semisubmerged on a nylon net. After 30 min, the sucrose buffer solution was replaced by an identical buffer solution except that NaCl was used instead of sucrose (equimolar substitution). Slices were perfused from below at a rate of ~1 ml/min, oxygenated with 95% O_2-5% CO_2 and warmed to 33–34°C. Recordings were made between 1.5 and 7 h after the dissection.

RECORDING AND STIMULATION. Recordings were made with borosilicate capillary-filled thin-walled glass (1.0 mm OD) pulled horizontally (model P-87, Sutter Instruments) to 2–5 MΩ (extracellular electrodes, filled with the saline-based buffer solution used to perfuse slices) or 60–100 MΩ (intracellular electrodes, filled with 1 M potassium acetate). Stimulation and recording sites are shown schematically in Fig. 2*A*. To examine evoked responses of the different cortical layers in vitro, serial recordings were made along an axis perpendicular to the pial surface (see next section for more information on localization of layers). For stimulation, a monopolar Teflon-coated stainless steel wire (75-μm diam) was placed on the border of layer VI and the white matter of the medial entorhinal cortex (''white matter'' stimulation) or in the center of either the presubiculum or parasubiculum (Fig. 2). Currents used for stimulation were monophasic rectangular pulses (50–100 μA, 10–100 μs). Stimulus frequency was 0.05–0.1 Hz unless stated otherwise. To standardize responses to white matter stimulation across slices, all responses in any given slice were evoked using a stimulus intensity that was just maximal for responses in layer VI of that slice. The maximal stimulus tested was 500 μA, 250 μs because stronger currents were not selective (i.e., there was current spread to other areas). To test responses to repetitive stimulation, a 1-Hz train of paired pulses (interstimulus interval, 40 ms) was triggered for 10 s (i.e., 10 pairs of stimuli). A stimulus strength was used that was approximately half the intensity which produced a maximal response.

For intracellular recording, an amplifier with a bridge circuit was used (Axoclamp 2A, Axon Instruments), and the bridge was balanced whenever current was passed through an intracellular electrode. Signals were digitized and stored on disk (Nicolet Model 410, Nicolet Instruments) or digitized and stored on tape (Neurocorder Model DR 484, Neurodata Instruments).

LOCALIZATION OF CORTICAL LAYERS IN VITRO. The localization of different cortical layers of slices during the experiment was based on the natural landmarks in the slice and aided by an ocular micrometer. The landmarks included the border between layer I and II, lamina dissecans, and the border of the white matter and layer VI, which are visible because of natural changes in opacity of the slice, and the visible differences between gray versus white matter. In AOAA-injected rats, the lamina dissecans became difficult to visualize probably because of the gliosis that occurs in that area (Du and Schwarcz 1995). Therefore only the white matter/layer VI border and the pia were used as landmarks. An ocular micrometer was used to position electrodes at specific distances from these landmarks, as follows: for layer I, 100–150 μm from the pial edge; layer II, 150–200 μm from the layer I/II border; layer III and V, 200 μm from the center of lamina dissecans; layer VI, 100–150 μm from the border of layer VI and the white matter (Fig. 2). Notably, the positions in layers II and III allowed us to avoid the layer II/III border, which moves to a slightly deeper site after AOAA treatment.

In vivo studies

RECORDING AND STIMULATION. Rats were anesthetized by subcutaneous urethan injection (1.25 mg/kg). If necessary, a supplemental intraperitoneal injection, ~15–30 min later, was used to achieve complete loss of reflexes (foot withdrawal in response to a toe pinch). Therefore the total dose was 1.25–1.5 mg/kg. Rats were placed in a stereotaxic apparatus (incisor bar, −3.3 mm). Animal temperature was maintained by a ho-

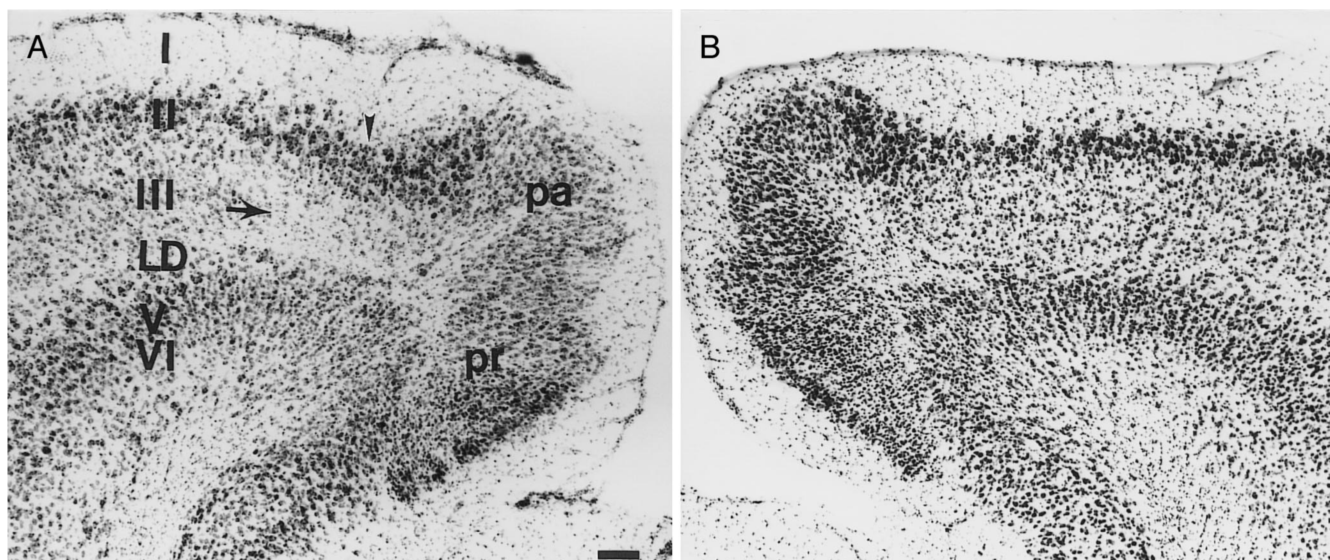

FIG. 1. Nissl-stained sections from amino-oxyacetic acid (AOAA)-treated and vehicle-injected (control) rats, 3 mo after treatment. *A*: section through a slice showing the entorhinal cortex of an AOAA-treated rat. This slice was taken from the same hemisphere where AOAA was injected 3 mo earlier. Note that there is cell loss in layer III of the medial entorhinal cortex (→) and that layer II is somewhat displaced, entering the former layer III (▲). This slice was used to record responses to white matter stimulation shown in Fig. 3. Dorsal is to the *left;* posterior is *up.* I, layer I; II, layer II; III, layer III; LD, lamina dissecans; V, layer V; VI, layer VI; pa, parasubiculum; pr, presubiculum. Scale bar for *A* and *B,* 100 μm. *B*: section through a slice from the contralateral hemisphere of the AOAA-treated rat used for *A.* Dorsal is to the *right;* posterior is *up.*

meothermic blanket with a rectal thermometer (Harvard Apparatus). Approximately 1–3 mm diameter holes were drilled in the skull over the entorhinal cortex and/or hippocampus of both hemispheres using a portable drill (Dremel). Skull holes over the entorhinal cortex were ~2 mm in diameter, and holes over the hippocampus were 2–4 mm in diameter. A bipolar stimulating electrode (modified from Rhodes NSC-100 so that tip separation was ~500 μm) was lowered perpendicularly, either in area CA3 (in mm: A-P, −3.3 to −3.7; M-L, 3.5–3.7, depth 3.0–3.5), area CA1 (A-P, −3.3 to −3.7; M-L, 2.0–3.0, depth 3.0–3.5), or the angular bundle (A-P, −7.8 to −8.4; M-L, 4.3–4.8, depth 3.0–3.5) of the left hemisphere. Stimulation of the right angular bundle also was tested in some experiments. An extracellular recording electrode was positioned in either the hippocampus or medial entorhinal cortex (left or right hemispheres, depending on the location of the stimulating electrode). Recording electrodes were lowered either at an angle or perpendicular to the animal. Figure 2*B* illustrates these sites schematically.

LOCALIZATION OF THE ENTORHINAL CORTEX IN VIVO. Recording sites in the entorhinal cortex were determined in the first experiments by examining the recording track in Nissl-stained sections (see *Anatomic methods*). The electrode track was most readily observed in sagittal sections (Fig. 8). After the initial experiments, it was clear that the recordings from the entorhinal cortex also could be used to determine its location because re-

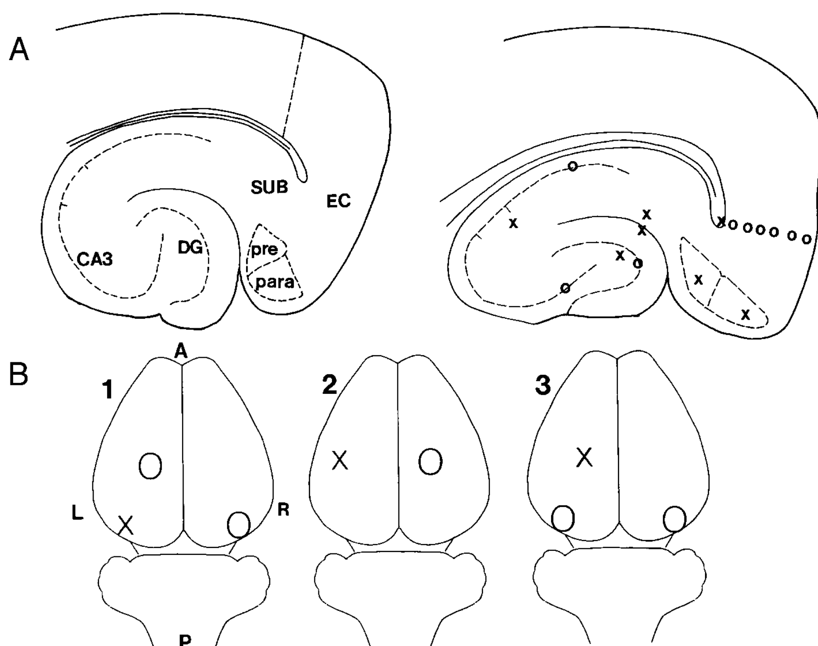

FIG. 2. Recording and stimulation sites for in vitro and in vivo experiments. *A*: recording and stimulation sites for in vitro experiments. Schematic diagrams of horizontal slices illustrate 2 of the different dorsal-ventral levels from which slices were taken. These diagrams are modified from Paxinos and Watson (1986). *Left*: section through the ventral hippocampus. – – –, cell layers of the hippocampus. DG, dentate gyrus; SUB, subiculum; EC, entorhinal cortex. Dotted lines surround the presubiculum (pre) and parasubiculum (para). *Right*: section at a more dorsal level. Recording (○) and stimulation sites (×) are shown. *B*: recording and stimulation sites for in vivo experiments. *1*: schematic diagram of the dorsal surface of the rat brain. A, anterior; P, posterior; L, left hemisphere, which was always the hemisphere in which AOAA was injected; R, right hemisphere. Recording (○) and stimulation sites (×) for responses to angular bundle stimulation. *2*: recording site in area CA1 (○) that was used to record responses to stimulation of the contralateral CA3 region (×). *3*: recording sites (○) used to test responses in the entorhinal cortex to stimulation of area CA1/subiculum (×).

sponses to area CA1 stimulation were large in this area (see RESULTS). Therefore, in subsequent experiments both Nissl-stained sections and electrophysiological responses were used to identify the recording site in the entorhinal cortex.

Anatomic methods

Immediately after in vitro recordings, slices were placed between filter paper, immersed in 4% paraformaldehyde, and fixed for >3 h. After in vivo recordings, an overdose of urethan was given and the brain was immersed in ~100 ml of 4% paraformaldehyde (pH 7.4) for 24 h on a rotator and then refrigerated, while still immersed, for another 24 h. Sections of slices or the whole brain (50-μm thick) were cut using a vibratome (Lancer) and transferred immediately to Tris buffer. Sections were stained with cresyl violet, dehydrated in a series of alcohols, cleared in xylene, and coverslipped using Permount (Fisher). Photomicrographs were made with a 35-mm camera attachment to an Olympus BH-2 microscope using Techpan film (Kodak).

Data analysis

Half-duration was used as an index of response duration instead of total duration because of the slow rate of decay of the residual part of some responses, making it difficult to pinpoint the exact time a response ended. Half-duration was defined as the time from the onset of the evoked response to the time during the decay of the response when amplitude was reduced to 50% of the maximum.

Measurements of theta amplitude were made at the site in the hippocampus where the negativity (extracellularly recorded EPSP) evoked by angular bundle stimulation was maximal (i.e., near the fissure). Amplitude was defined as the millivolt change from the peak to the trough of a cycle (oscillation). For each animal, a value was calculated from averaging the amplitude of 10 contiguous oscillations. The period of 10 oscillations was taken ≥2 min after an angular bundle stimulus.

The population EPSP in the dentate gyrus was defined as the positivity on which the population spike is superimposed and was measured from baseline to peak. The population spike amplitude was calculated as the average voltage change of the negative- and positive-going phases of the population spike (Alger and Teyler 1983; Scharfman 1997). Paired-pulse inhibition and facilitation measurements were based on stimuli that were half-maximal. Paired-pulse inhibition (PPI; Table 1) was calculated as (population spike amplitude in response to the second stimulus)/(population spike amplitude evoked by the first stimulus), and is expressed as a percent in Table 1.

Data are expressed means ± SE. A Student's *t*-test was used to evaluate statistical differences between means. The *P* value was set at 0.05 prior to all experiments.

RESULTS

Entorhinal cortex

IN VITRO STUDIES. In seven AOAA-treated rats, 37 slices were examined. Three of the seven rats had received an AOAA injection 1.5 yr previously, and the other four had been treated 1–6 mo earlier. Data were pooled because the results were indistinguishable, and Nissl-staining demonstrated a comparable lesion, affecting layer III of the medial entorhinal cortex preferentially (Fig. 1). There was no detectable cell loss in the entorhinal cortex of the contralateral hemisphere of AOAA-injected rats, as has been reported (Du and Schwarcz 1992; Du et al. 1998) (Fig. 1).

The data from AOAA-treated rat slices were compared with 19 slices from four control rats, two of which were littermates to the older AOAA-treated group and had been injected with vehicle instead of AOAA. The other two were littermates to the younger group and were injected with phosphate-buffered saline instead of AOAA. Results of the controls were pooled because they were indistinguishable.

TABLE 1. *Comparison of afferent responses recorded in hippocampus in AOAA-treated rats and controls in vitro*

	Control	AOAA
Dentate gyrus		
Population EPSP amplitude, mV	5.9 ± 0.30	5.6 ± 0.21
Population spike amplitude, mV	3.2 ± 0.66	2.8 ± 0.34
Paired-pulse inhibition 20 ms ISI, %	18.3 ± 6.9	11.8 ± 4.1
n	20	11
Area CA1		
Population spike amplitude, mV, Schaffer collateral stimulation	12.1 ± 0.41	14.2 ± 0.43
Population spike amplitude, mV, stratum lacunosum-moleculare stimulation	11.4 ± 0.51	10.8 ± 0.32
Paired pulse inhibition 20 ms ISI, %	65.2 ± 8.1	60.2 ± 5.2
n	13	8

Values are means ± SE. Stimulus intensity was half the intensity that produced a maximal response. Sample sizes refer to the number of slices tested from the left hemisphere (hemisphere ipsilateral to the AOAA injection). Recording and stimulation sites are shown in Fig. 2. Amplitude calculations are described in METHODS. ISI, interstimulus interval. Paired-pulse inhibition is based on the ratio of the second response amplitude to the first and is expressed as a percent (see METHODS). None of the differences between control and AOAA rats were statistically significant ($P > 0.05$; Student's *t*-test).

Three afferent responses were evaluated in entorhinal cortical neurons, in slices from both hemispheres, by stimulating the white matter, pre- or parasubiculum (Fig. 2). Responses to stimulation of these sites were recorded throughout the layers of the medial entorhinal cortex.

White matter stimulation. Stimulation of the white matter in naive rats produces a stereotypical pattern of responses in the different cortical layers that can be recorded extracellularly, and is similar qualitatively from slice to slice (Fig. 3, Con) (see Scharfman 1996). In the majority of the 31 slices from AOAA-treated rats in which the entorhinal cortex was examined, these responses differed from controls, in one of two ways.

First, responses that were evoked in the superficial layers were prolonged and contained repetitive components, indicative of hyperexcitability (Fig. 3, AOAA; $n = 11$ of 31 slices). The long duration of the responses was evident in the difference in half-duration. In the AOAA-treated slices, the half-duration of responses recorded in layer III was >100 ms ($n = 11$), but it was <30 ms in controls ($n = 19$). Note that in many AOAA-treated rats, layer III appeared to shrink and layer II moved into the area where layer III ordinarily would be located (Fig. 1). Therefore it is likely that some of the responses that were recorded in ''layer III'' of AOAA-treated rat slices reflected the activity of layer II somata more than control rat slices. To minimize this difference, layer III recording sites were in the deep part of layer III (see METHODS).

A second type of abnormality was the small response amplitude (<1-mV peak amplitude) in the area corresponding to layers I–III ($n = 8$ of 31 slices). In these same slices,

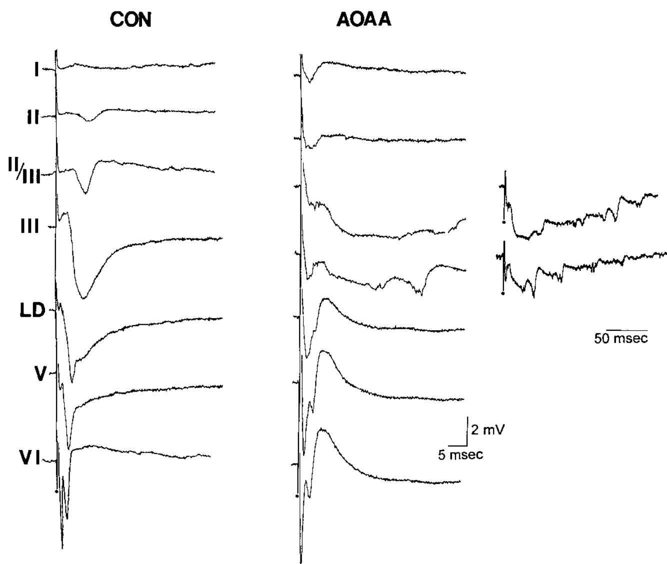

FIG. 3. Extracellularly recorded evoked responses to white matter stimulation, recorded in medial entorhinal cortex in slices from AOAA-treated rats and controls. The responses of entorhinal neurons recorded extracellularly from layers I–VI are shown. The stimulation site was on the border of layer VI and the white matter in the medial entorhinal cortex. A stimulus intensity was chosen that evoked the maximum response in layer VI. *Left*: responses are shown that were recorded from slices of a vehicle-injected rat. These responses are similar to those recorded from naive rats (Scharfman 1996). Stimulus artifacts are marked (●). LD, lamina dissecans. *Right*: responses are shown that were recorded from slices of an AOAA-treated rat. Note that responses recorded in layer III contained repetitive activity (responses recorded from layer II/III and layer III are shown in their entirety at *far right* using a different time scale). Responses recorded in deep layers and in layer I did not contain repetitive activity. A similar, lamina-specific pattern was observed in other AOAA-treated rats (see text).

responses evoked in deeper layers were normal qualitatively but were also small in amplitude (<1 mV, baseline to peak for responses in layers V–VI). These reduced responses are consistent with cell loss in the entorhinal cortex of AOAA-treated rats but could also be due to other factors (see DISCUSSION).

The other 12 slices of the 31 that were examined were not distinguishable from control slices in their responses to white matter stimulation. Notably, there could be variability among slices from the same AOAA-treated rat. One slice could demonstrate abnormal responses, whereas another slice from the same animal could be relatively normal, even though they were treated identically (e.g., juxtaposed in the recording chamber, sliced at the same time, transferred to the recording chamber at the same time, etc.).

Consistent with the possibility that prolonged excitation of entorhinal neurons occurred during the prolonged evoked responses in some of the slices (described in the 2nd paragraph of this section), intracellular recordings from the same slices demonstrated that neurons recorded from superficial layers produced complex depolarizations in response to white matter stimulation, even when low stimulus intensities were tested (i.e., below the intensities used to evoke the field potentials shown in Fig. 3; in all of the 10 layer III cells tested; Fig. 4). These depolarizations appeared to be composed of multiple EPSPs because they increased in amplitude with hyperpolarization of the cell (data not shown). Furthermore, action potentials occurred at the peak of the depolarizations (Fig. 4). These types of complex excitatory responses were not recorded from neurons in any of the control slices (n = 15 neurons in layer III).

Many neurons that were recorded in deeper layers (n = 4 of 8 in layer V; n = 2 of 2 in layer VI) in the same slices where superficial cells were abnormal (as described earlier) responded to stimulation with relatively simple depolarizations and single discharge, similar to neurons in deep layers of control slices. However, an abnormal response was recorded from the others (4 of 8 neurons in layer V; Fig. 4). This response began normally, i.e., a monophasic EPSP and single discharge was evoked after a brief latency (<3 ms from stimulus artifact to the onset of the EPSP; Fig. 4). At a latency of 50–100 ms after the stimulus, a barrage of EPSPs and discharges occurred (Fig. 4). The barrages lasted ~100 ms. The four layer V neurons with these responses were recorded in three different slices from two different AOAA-treated rats (both of these rats were <6 mo post-AOAA injection). These responses indicate that additional polysynaptic excitatory circuits may have been present in these slices, perhaps reflecting synaptic reorganization following AOAA-induced cell loss (see DISCUSSION).

Stimulation of pre- or parasubiculum. Stimulation of the presubiculum in slices from control rats produced a negative potential that was maximal in layer III (Fig. 5). This is consistent with the known pathway from the presubiculum that terminates in layer III preferentially (Caballero-Bleda and Witter 1993; Eid et al. 1996; Köhler 1984; Van Groen and Wyss 1990). Stimulation of the parasubiculum in the same slices produced a short-latency negativity that was maximal in layer II (Fig. 5). This response is likely to reflect the known input to layer II neurons from the parasubiculum

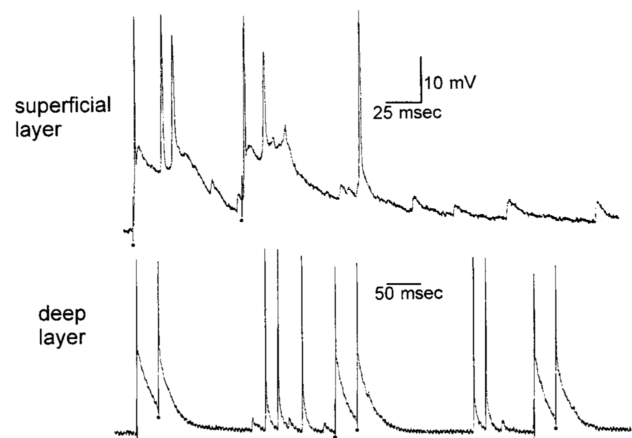

FIG. 4. Evoked responses recorded intracellularly from medial entorhinal cortical neurons in slices from AOAA-treated rats. *Top*: in slices from AOAA-treated rats, responses of neurons recorded in superficial layers to white matter stimuli consisted of repetitive depolarizations, as in this example from a neuron recorded in layer III. A pair of stimuli was triggered with a 100-ms interstimulus interval. Stimulus artifacts are marked (●). *Bottom*: in the same slice as the neuron whose response is shown in *A*, neurons recorded in deep layers demonstrated different evoked responses. Their responses were similar to neurons in control slices, where white matter stimulation evoked a large excitatory postsynaptic potential (EPSP) and single action potential at a short latency after a stimulus. However, in a subset of neurons, this brief response was followed by a barrage of EPSPs and action potentials. An example of such a response is shown. Pairs of stimuli, 50 ms apart, were triggered 3 times. Between the pairs of stimuli repetitive EPSPs and action potentials occurred.

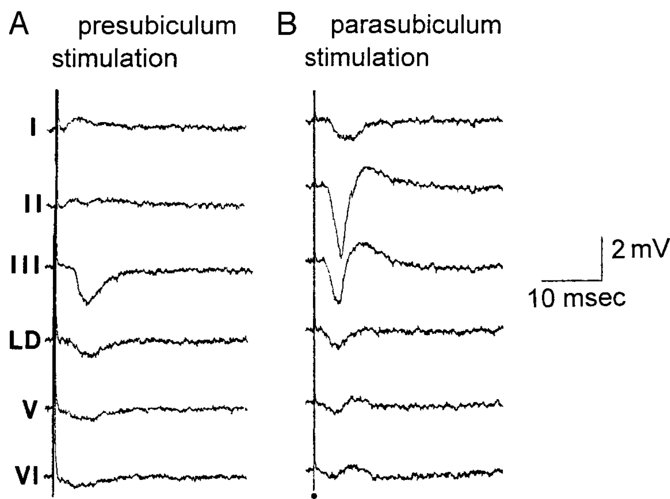

FIG. 5. Extracellularly recorded evoked responses to pre- and parasubicular stimulation in control rat slices. *A*: responses to presubiculum stimulation of medial entorhinal cortex are shown (see Fig. 1 for stimulation and recording sites). A stimulus intensity was chosen that produced a maximal response in layer III, which was the layer where the largest response was always recorded. This result is consistent with the selective projection of presubicular neurons to layer III (Caballero-Bleda and Witter 1993; Eid et al. 1996; Köhler 1984; Van Groen and Wyss 1990). Note that in AOAA-treated rats no responses were evoked by presubiculum stimulation. LD, lamina dissecans. Stimulus artifacts are marked (●). *B*: responses to parasubiculum stimulation of medial entorhinal cortex are shown (see Fig. 1 for stimulation and recording sites). A stimulus intensity was chosen that produced a maximal response in layer II, where the largest response was always recorded. This result is consistent with the known preferential projection of the parasubiculum to layer II (Van Groen and Wyss 1990).

(Van Groen and Wyss 1990). In seven of eight slices from AOAA-treated rats, no responses could be recorded in any layer of the medial entorhinal cortex in response to either pre- or parasubiculum stimulation. The lack of response could be due to a loss of the neurons or neuronal elements in the superficial layers that produce the evoked responses, or changes in the presubiculum or parasubiculum (see DISCUSSION).

Evoked responses in slices ipsilateral and contralateral to the lesion. Responses to pre- and parasubiculum stimulation in slices taken from the hemisphere contralateral to the lesion were similar to responses evoked in slices from control rats ($n = 4$ slices). However, responses to white matter stimulation in the contralateral slices were abnormal in six of eight slices tested. Responses recorded in these six slices were similar to the abnormally prolonged, repetitive ipsilateral responses (described above and see Fig. 3). Neurons recorded intracellularly in superficial layers of these slices responded to white matter stimulation with prolonged, complex depolarizations, like neurons from the superficial layers of ipsilateral slices ($n = 4$; Fig. 4). Extracellularly recorded responses in deeper layers were not abnormal, indicating further similarity between ipsilateral and contralateral entorhinal neurons.

IN VIVO STUDIES. The in vitro data described in the previous section showed that abnormal synaptic responses were not present in all slices examined from the lesioned hemisphere. This could be due to the small size of the lesion, as well as other factors (see DISCUSSION). However, it also could be due to the fact that slices allow only a portion of the entorhinal cortical circuitry to be examined. To determine if more robust epileptiform activity would be present in vivo, recordings were made in anesthetized AOAA-treated rats ($n = 6$) and compared with vehicle-injected controls ($n = 4$) or animals which were not injected at all (naive controls, $n = 4$). There were no detectable differences among the vehicle-injected rats and rats which were not injected, so they are referred to collectively as controls.

Entorhinal responses to area CA1/subiculum stimulation in control rats. Stimulation at the border of CA1 and the subiculum was used to test responses of entorhinal neurons to afferent stimulation because of the known termination site of the perforant path in stratum lacunosum-moleculare of CA1 (Hjorth-Simonsen and Jeune 1972; Steward 1976; Steward and Scoville 1976; Wyss 1981), the projection of the entorhinal cortex to the subiculum (Behr et al. 1998; Van Groen and Lopes da Silva 1986) and of the subiculum to the entorhinal cortex (Van Groen and Lopes da Silva 1986; see also Lopes da Silva et al. 1990; Witter 1993 for review). Thus this site probably activated the entorhinal cortex antidromically and orthodromically.

Initial experiments examined responses of the right entorhinal cortex to stimulation of the left CA1/subiculum region. Responses to this stimulation site were robust and varied little from animal to animal in naive controls ($n = 4$). An example of responses from a vehicle-injected rat is shown in Fig. 6; recording and stimulation sites were confirmed by Nissl staining (e.g., Figs. 7 and 8). Consistent with the known location of perforant path terminals in stratum lacunosum-moleculare of the hippocampus, responses were not evoked unless the stimulating electrode was at a depth corresponding to that layer. Responses were similar when the stimulating electrode was positioned deeper, in the outer two-thirds of stratum moleculare of the dentate gyrus, again consistent with the location of perforant path terminals there, but decreased at greater depths. This pattern indicates that responses were probably due, at least in part, to antidromic activation of entorhinal neurons. Indeed, an extremely short latency (<2 ms time to onset), i.e., a putative antidromic potential, could be evoked in the ipsilateral entorhinal cortex of control rats in response to such CA1 stimulation.

A representative series of responses recorded from the right entorhinal cortex in response to left CA1 stimulation in a control rat are shown in Fig. 6. A large negativity, which ranged in latency from 8 to 12 ms, was evoked at relatively superficial depths, and this changed polarity as the recording depth increased. An inflection was present on the positive potential recorded at the deeper sites, indicative of a population spike. However, without intracellular recordings it is not certain that a population spike underlies this event. Because the recording sites corresponded to an electrode track passing first through layer III and subsequently layer II (Fig. 8), a parsimonious explanation of these recordings is that the negative potential recorded initially (at superficial sites in the track) represented extracellularly recorded EPSPs on the dendrites of cortical neurons located in layer III (apical dendrites of layer V/VI pyramidal cells and basal dendrites of layer II pyramidal cells). The positive potential observed at deeper sites in the track could represent the reversal of the extracellularly recorded EPSPs. The putative population

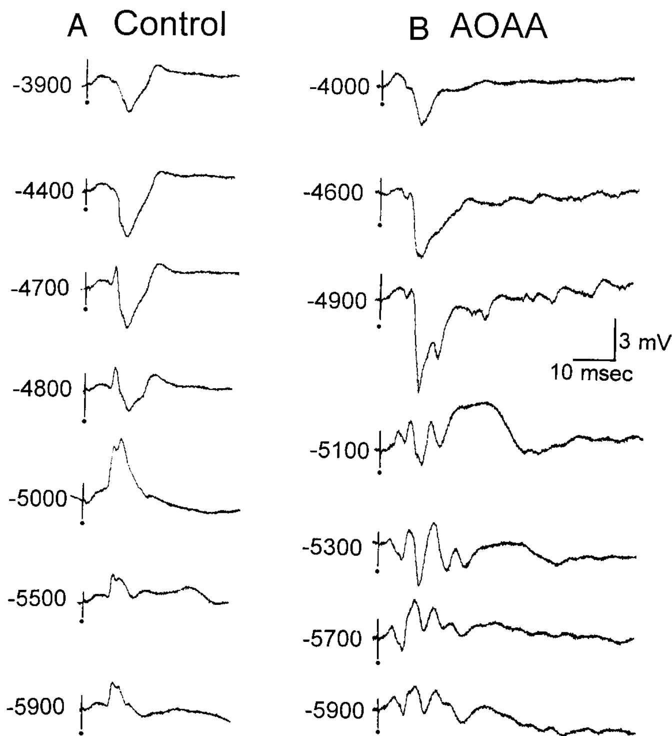

FIG. 6. Extracellularly recorded responses to stimulation of area CA1 of the left hemisphere recorded in the contralateral entorhinal cortex in vivo. Responses to stimulation of the CA1/subiculum border were recorded at several depths in the contralateral entorhinal cortex. A maximal stimulus intensity was used for each stimulus. Recordings began at 4,000 μm below the surface of the brain and continued in 200-μm increments to the deepest site. Stimulus artifacts are marked (●). *Left*: evoked responses in a vehicle-injected rat demonstrate a prominent negativity superficially that flips polarity at deeper sites. *Right*: evoked responses in an AOAA-treated rat demonstrate more complex waveforms that indicate longer duration and repetitive responses. Such responses support the results of in vitro data, which indicated that the entorhinal cortex was hyperexcitable in AOAA-treated rats but not in vehicle-injected controls.

spike recorded at deep sites could represent the summation of action potentials produced in layer II neurons that were reached as the recording electrode passed into the layer II cell layer.

Responses to CA1/subiculum stimulation in AOAA-treated rats. In AOAA-treated rats, the responses to left CA1/subiculum stimulation of the right entorhinal cortex were prolonged and more complex ($n = 4$ of 5 rats; Fig. 6). These data indicate that the responses evoked in the contralateral entorhinal cortex in these rats were abnormal. Given the likelihood that some of the fibers contributing to this response originated in the subiculum (Van Haeften et al. 1995) and that the subicular input to the entorhinal cortex contains an *N*-methyl-D-aspartate (NMDA)-receptor-mediated component (Jones 1987), this result may reflect an enhanced NMDA-receptor-mediated response to subicular stimulation in AOAA-treated rats. This is consistent with the known effects of AOAA and entorhinal seizures to increase NMDA-receptor-mediated synaptic responses in the entorhinal cortex (Bear et al. 1996; Eid et al. 1995; Scharfman 1996).

In addition to testing responses to single stimuli, 1-Hz stimulation (for 3–6 s) produced activity that persisted after

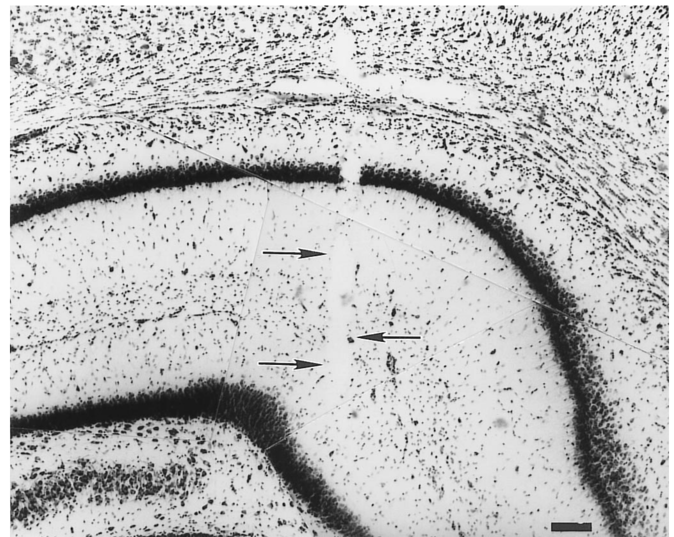

FIG. 7. Location of area CA1/subiculum stimulation site used for in vivo activation of the entorhinal cortex. Stimulation site used for the recordings shown in Fig. 6 is illustrated in a montage of adjacent Nissl-stained sections from the brain that was used for that experiment. Note the track made by the stimulating electrode (→) passes into area CA1 near the subiculum. Only 1 pole of the electrode is shown in this section; the other pole was in a similar area in a more anterior section. Scale bar, 100 μm.

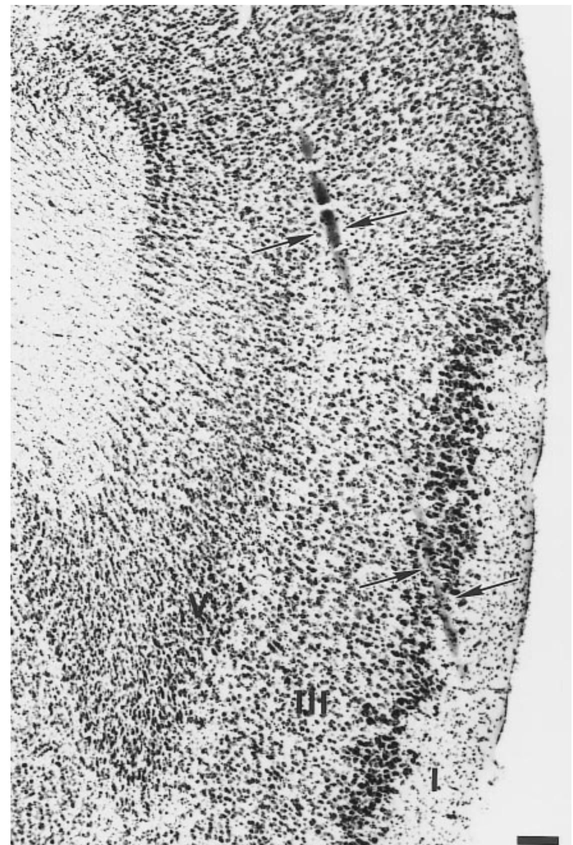

FIG. 8. Localization of recording sites in the entorhinal cortex in vivo. Photomicrograph shows a recording electrode track through the entorhinal cortex after immersion fixing the brain so that blood collected in the track. Sagittal section from the brain is shown with the electrode track indicated (→). As the recording electrode was lowered it passed from layer III into layer II and finally into layer I. Dorsal is *up,* posterior is to the *right.* Scale bar, 100 μm.

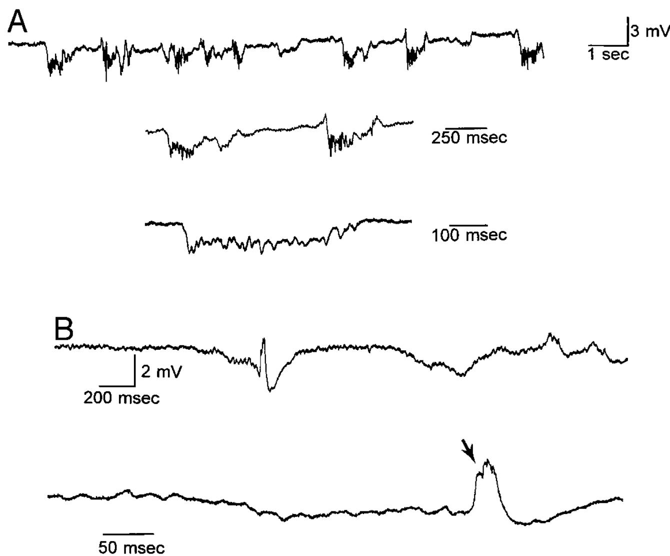

FIG. 9. In vivo spontaneous activity recorded in the entorhinal cortex and hippocampus of AOAA-treated rats and controls. *A*: spontaneous activity recorded extracellularly from the entorhinal cortex of an AOAA-treated rat is shown with three different time scales. *B*: spontaneous activity recorded extracellularly from the right hippocampus of an AOAA-treated rat is shown with 2 different time scales. This activity was recorded near the pyramidal cell layer, so the small deflection superimposed on the large positivity (→) is likely to be a small population spike.

stimulation ended (Fig. 9). Notably, this did not occur in control rats and did not occur if the white matter was stimulated analogously in vitro. This activity consisted of prolonged negative DC shifts (1–2 mV amplitude) lasting >100 ms, and increased noise or repetitive undulations, similar to epileptiform activity (Fig. 9). The activity persisted for several minutes. When recordings were made in the hippocampus during this time, spontaneous DC shifts also occurred there (Fig. 9), indicating abnormal hippocampal activity. However, the hippocampal activity was only observed in two of four rats where it was examined. The activity in the entorhinal cortex occurred in four of five rats tested.

Surprisingly, evoked responses of the left entorhinal cortex to stimulation of the right CA1/subiculum region were inconsistent (note that these responses were tested before insertion of an angular bundle stimulating electrode). In five of five rats examined, numerous recording sites in the ipsilateral hemisphere were used and were confirmed to be in the entorhinal cortex, but the responses were small and variable. Spontaneous activity was not present after 1-Hz stimulation. This lack of effect could be due to the loss of neurons in the ipsilateral hemisphere, or other factors (see DISCUSSION).

Hippocampus

IN VITRO STUDIES. The same slices in which the entorhinal cortex was examined also were used to test responses of hippocampal neurons to afferent stimulation. Fifty-two slices from seven AOAA rats were compared with 26 slices from four control rats. In AOAA-treated rats, 45 slices were from the hemisphere ipsilateral to the AOAA-induced lesion (left hemisphere) and 7 were from the contralateral side. In control rats, 20 slices were from the left hemisphere and 6 were from the right side.

Three pathways were tested (Fig. 2). Responses to outer molecular layer stimulation were recorded from the granule cell layer/hilar border to assess the perforant path input to granule cells. To examine the mossy fiber input to CA3 pyramidal cells, responses of CA3 pyramidal cells to hilar stimulation were recorded from the CA3b/c cell layer. Area CA1b pyramidal cell responses to Schaffer collateral stimulation were tested by stimulating in stratum radiatum of CA2. Responses recorded at the same CA1b site also were examined after stimulation of stratum lacunosum-moleculare with the stimulating electrode positioned near the subiculum. All stimulation and recording sites are illustrated in Fig. 2.

Activation of area CA3 by mossy fiber stimulation. In 11 of 15 slices examined ipsilateral to the AOAA-induced lesion, and 4 of 6 slices contralateral to the lesion, hilar stimulation (tested using half-maximal intensity) evoked multiple population spikes to a single stimulus or paired stimuli, 40 ms apart (Fig. 10). In three slices, spreading depression followed stimulation at 1 Hz for <5 s. This did not occur in any of the eight slices from control rats, even if maximal stimulus strength, twice the frequency (2 Hz), and more than twice the number of stimuli were tested. These data suggest that AOAA treatment produced a change, either direct or indirect, in area CA3 responses to mossy fiber stimulation (see DISCUSSION).

Outer molecular layer stimulation of granule cells. Responses of granule cells to outer molecular layer (perforant path) stimulation in 21 slices from AOAA-treated rats were similar to those recorded from 14 slices of control rats. These slices were primarily from the hemisphere ipsilateral to the lesion ($n = 20$ ipsilateral slices and 1 contralateral in AOAA rats; $n = 11$ slices from the left hemisphere and 3 from the right hemisphere in control rats). Population spike and population EPSP waveforms were indistinguishable in AOAA-treated tissue and control slices and did not indicate

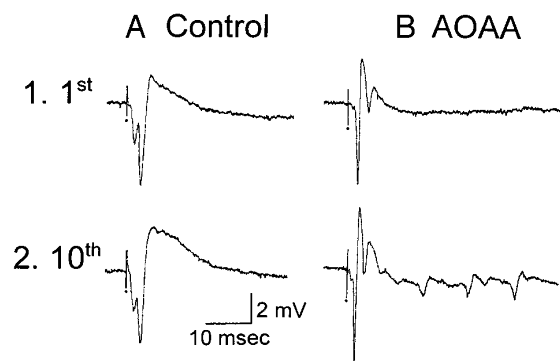

FIG. 10. Responses of CA3 pyramidal cells to hilar stimulation, recorded extracellularly in AOAA-treated rats and controls in vitro. *A*: responses to hilar stimulation were recorded in the area CA3 pyramidal cell layer (CA3b/c subfield) of a vehicle-injected animal, 1.5 yr after vehicle-injection. *1*: response to the 1st stimulus of a 1-Hz train is shown. A stimulus intensity was used that evoked a half-maximal response. Stimulus artifacts are marked (●) and are clipped. *2*: response to the 10th stimulus is shown. *B*: responses to hilar stimulation were recorded in area CA3b/c as in *A*, in a rat that was injected with AOAA 1.5 yr earlier. *1*: response to the 1st stimulus is shown. *2*: response to the 10th stimulus is shown. Note that multiple population spikes were evoked. There were multiple population spikes evoked by every stimulus after the 3rd stimulus of the train.

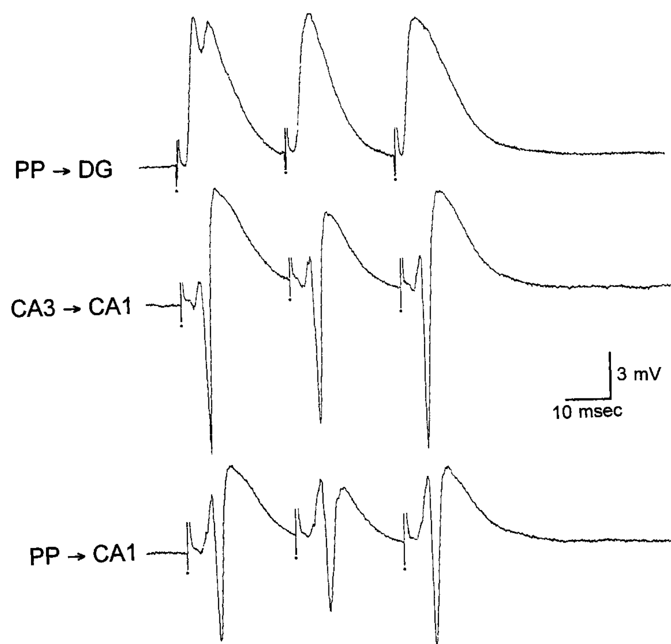

FIG. 11. Responses to paired-pulse stimulation of the trisynaptic pathway of hippocampal principal cells in slices from AOAA-treated rats. *Top row*: 3 responses are overlapped. Each was a response to outer molecular layer stimulation that was recorded at the border of the granule cell layer and the hilus as shown in Fig. 2. *Left*: response to a single stimulus using an intensity that evoked a half-maximal response. *Center*: 2nd response of a pair of stimuli, 20-ms interstimulus interval. Same stimulus intensity as at left. *Right*: 2nd response of a pair of stimuli, 30-ms interstimulus interval. Same stimulus intensity as at left. Note that strong paired-pulse inhibition is evident, as in control slices. Stimulus artifacts are clipped and marked by dots. PP, perforant path; DG, dentate gyrus. *Middle row*: responses to stimulation of the Schaffer collaterals of CA3 pyramidal cells were recorded in the CA1 pyramidal cell layer as shown in Fig. 2. Pairs of stimuli were triggered, analogous to the responses recorded from the dentate gyrus, above. *Bottom row*: responses to stratum lacunosum-moleculare stimulation were recorded from the CA1 pyramidal cell layer. The stimulus site was near the subiculum in stratum lacunosum-moleculare (see Fig. 2). Pairs of stimuli were triggered as for the *top* and *middle* traces.

hyperexcitability because there was only one population spike in response to a single stimulus and there was strong paired-pulse inhibition (Fig. 11; Table 1). Maximal population EPSP amplitude and population spike amplitude were not significantly different (Student's *t*-test; $P > 0.05$; Table 1). The difference in percent paired-pulse inhibition using 20-ms interstimulus intervals was not significant (Student's *t*-test; $P > 0.05$; Table 1). The inhibitory effects of 1-Hz stimulation on population spike amplitude that are typical of control rat slices (frequency-dependent depression) (Burdette and Masukawa 1995; Sloviter 1991) also were recorded in slices from AOAA-treated rats.

Responses of CA1 pyramidal cells to afferent stimulation. Responses of area CA1 pyramidal cells to Schaffer collateral or stratum lacunosum-moleculare stimulation (Fig. 11) showed no evidence of hyperexcitability in waveform, number of population spikes, or paired pulse-inhibition/facilitation (Fig. 11; $n = 15$ AOAA-treated slices, 13 ipsilateral to the lesion and 2 contralateral vs. 12 control slices, 8 from the left hemisphere and 4 from the right hemisphere). Maximal population spike amplitude and paired-pulse inhibition (20-ms interstimulus interval) were not significantly different (Student's *t*-test; $P > 0.05$; Table 1). Moreover, both AOAA-treated rats and control rats required a higher stimulus strength to evoke a population spike by the stratum lacunosum-moleculare input than the Schaffer collateral input. Stimulation at 1 Hz did not evoke multiple population spikes, although it did in vivo (see next section).

IN VIVO STUDIES. *Perforant path input to the hippocampus.* To test responses of granule cells to angular bundle stimulation in vivo, both recording and stimulating sites were in the left hemisphere, ipsilateral to the AOAA-lesion (Fig. 2). Consistent with the in vitro studies described in the previous section, stimulation of the angular bundle in vivo evoked responses in the hippocampus that were indistinguishable from control rats. Figure 12 illustrates responses evoked at various depths in the hippocampus in response to a fixed stimulus to the angular bundle (for comparative purposes, a stimulus strength was chosen in each animal that evoked a small population spike in the granule cell layer). Note that in Fig. 12 there are potentials that occur at long latencies that differ somewhat between the AOAA-treated rat and control rat; these differences were not reproducible.

Although evoked responses did not appear to differ among AOAA-treated and control rats, there was a difference in spontaneous activity recorded in the dentate gyrus. In control rats, a prominent theta rhythm was present in stratum lacunosum-moleculare and the outer molecular layer and was particularly large at the same recording depth where the maximum negativity was evoked by angular bundle stimulation (Fig. 12). This is likely to reflect the entorhinal cortical component to theta rhythm (for recordings of theta in the entorhinal cortex, see Alonso and Garcia-Austt 1987; Mitchell and Ranck 1980; for hippocampal recordings of theta, see Brankack et al. 1993; Buzsaki et al. 1983; Leung 1998; Vanderwolf and Leung 1983). Fast oscillations similar to those reported previously (Bragin et al. 1995) were recorded at deeper sites, where the population spike and associated positivity were maximal (i.e., the granule cell layer/hilar border and in the hilus; Fig. 12). In AOAA-treated rats, theta amplitude was reduced and less robust near the hippocampal fissure (mean maximal amplitude, AOAA-treated rats, 1.5 ± 0.1 mV; control rats, 0.5 ± 0.2 mV; Student's *t*-test, $P < 0.05$; Fig. 12). In addition to the reduction in theta, there were disruptions of the fast oscillations at the granule cell layer/hilar border and in the hilus (Fig. 12).

CA3 stimulation of area CA1. A previous report described that commissural stimulation of CA1 in AOAA-treated rats was abnormal in that more than one population spike occurred in response to paired stimulation (Denslow et al. 1995). We did not find evidence in vitro that responses of CA1 neurons were abnormal, so in vivo experiments were conducted to determine if the different experimental preparations might underlie the difference between the in vitro and in vivo results.

To study the afferent responses of CA1 neurons in vivo, area CA3 of the left hemisphere (ipsilateral to the lesion) was stimulated, and recordings were made in the contralateral CA1 pyramidal cell layer (Fig. 13). This stimulation site is likely to activate CA3 axons and their input to CA1 as well as other commissural pathways. We found that single population spikes in area CA1 were evoked in response to

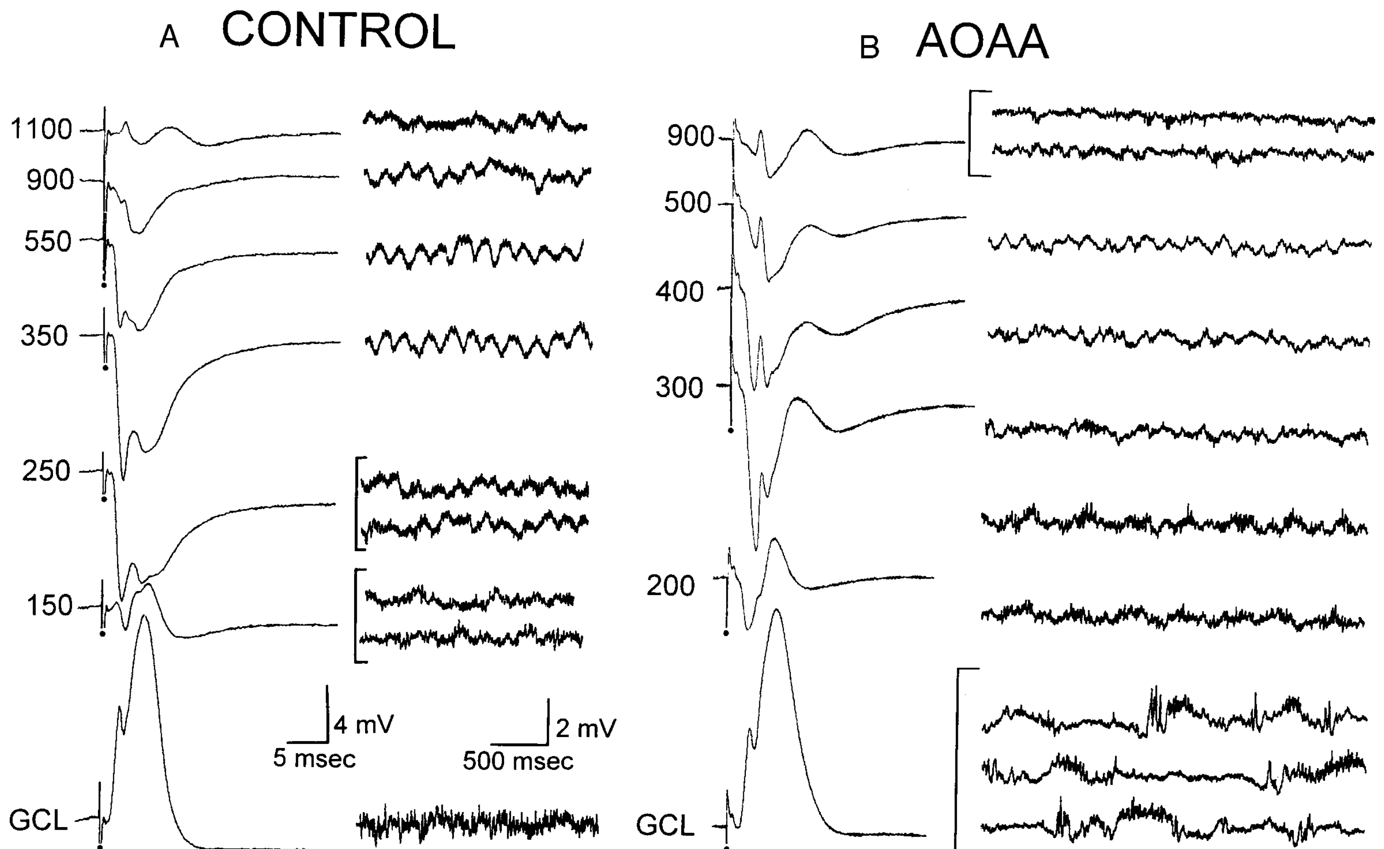

FIG. 12. Spontaneous rhythms in the dentate gyrus of AOAA-treated rats and controls. *A*: recordings from a vehicle-injected rat. *Left*: evoked responses to angular bundle stimulation. Numbers beside traces are distances from the granule cell layer, in micrometers. The granule cell layer was defined as the site where the maximal populaton spike could be recorded. Stimulus strength was half-maximal. *Right*: spontaneous activity recorded in the same location as the angular bundle response on the left. More than 1 sample of spontaneous activity is shown for the recording sites 150 and 250 μm from the granule cell layer. *B*: recordings from an AOAA-treated rat. Recordings are shown that were made in an analogous fashion to those in *A*. Note that at the level of the granule cell layer, the spontaneous activity is irregular in the AOAA-treated rat. In addition, theta rhythm observed at the depths corresponding to the outer molecular layer and stratum lacunosum-moleculare is smaller in the AOAA-treated rat.

a single contralateral CA3 stimulus in the majority of experiments. However, repetitive stimulation (1-Hz paired stimuli for 2–5 s, 40–70 ms interstimulus interval; see METHODS) evoked multiple population spikes (Fig. 13). Multiple population spikes were not observed in control rats in vivo or in AOAA-treated rats in vitro (Fig. 13). We interpret these results as evidence that there was greater excitability in AOAA-treated rats compared with controls in the CA3/commissural pathway input to contralateral CA1 pyramidal cells.

There also were other differences from control rats in the synaptic responses recorded in vivo. As shown in Fig. 13, 1-Hz stimulation (paired stimuli, 70-ms interstimulus interval, using half-maximal stimulus strength, for 10 s), potentiated evoked responses in both AOAA-treated (mean 165% of control) and control rats (151% of control). The percentages refer to the increase in amplitude of the first population spike not the later ones. Potentiation of the first population spike lasted much longer in controls than in AOAA-treated rats. In three of three control rats, potentiation lasted >10 min. However, in four of four AOAA-treated rats, the same train led to potentiation that never exceeded 30 s. Thus repetitive stimulation had very different effects in area CA1: in control rats it *did not* lead to multiple population spikes but *did* lead to long-lasting potentiation; in AOAA-treated rats it *did* lead to multiple population spikes but *did not* lead to long-lasting potentiation.

DISCUSSION

Summary

The primary finding of this study is that there was evidence of hyperexcitability in the hippocampus and entorhinal cortex several months and even >1 yr after AOAA treatment. Both the hemisphere ipsilateral and contralateral to the lesion were affected, although Nissl staining revealed evidence of anatomic changes only in the ipsilateral side. The results suggest that a unilateral lesion of the superficial layers of the entorhinal cortex can lead to lasting changes in excitability in limbic areas. The data support the growing evidence that the entorhinal cortex plays an important role in controlling activity in hippocampal and parahippocampal circuits (Collins et al. 1983; Heinemann et al. 1992). They also indicate that the cells of origin of the perforant path, and specifically neurons in layer III, may be particularly important in this regard.

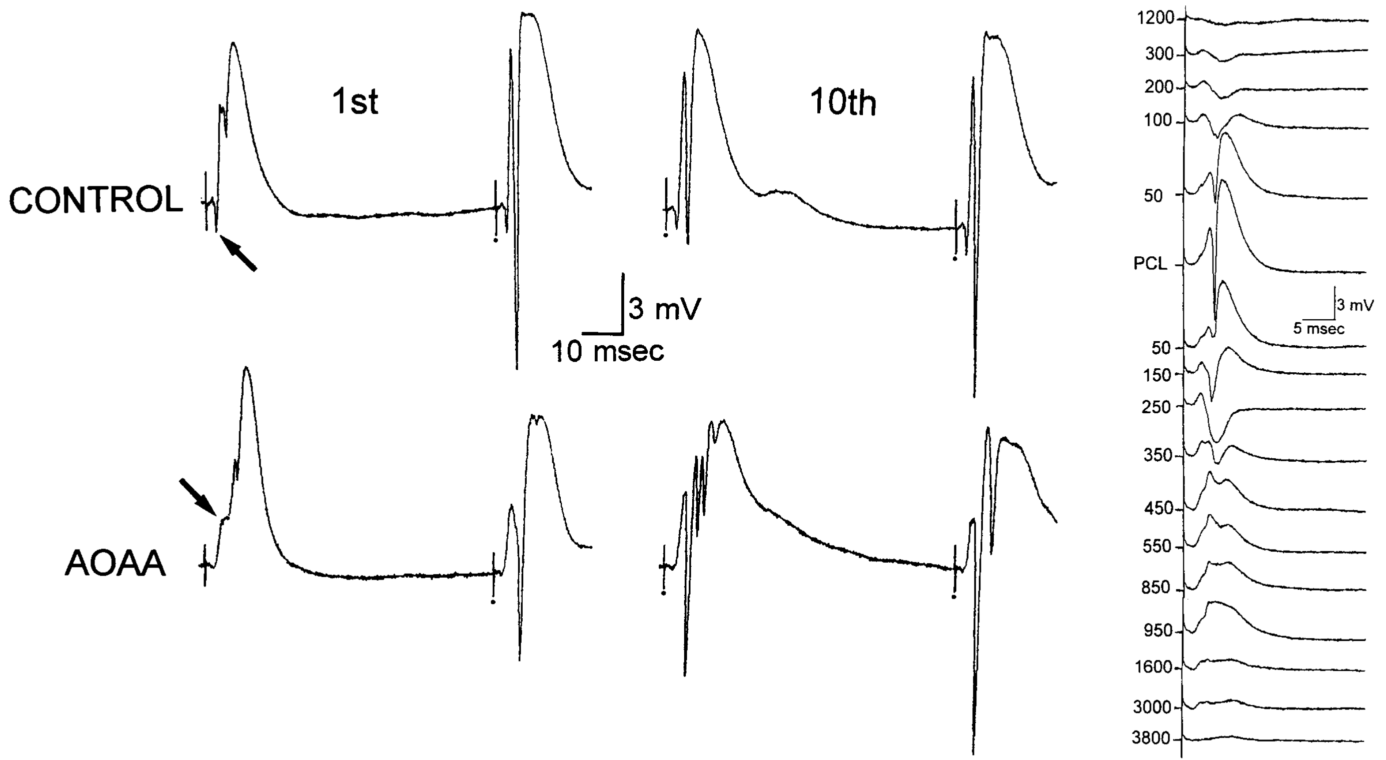

FIG. 13. Responses of area CA1 pyramidal cells in vivo to stimulation of contralateral area CA3 in AOAA-treated rats and controls. Responses to stimulation of area CA3 of the left (lesioned) hemisphere were recorded in area CA1 of the right hemisphere. Stimulus intensity was adjusted to produce a small population spike in response to the 1st stimulus. Confirmation of stimulation and recording sites were made as described in the METHODS (and see Scharfman and Goodman 1998). ●, stimulus artifacts, which are clipped. *Top*: responses to paired stimulation (70-ms interstimulus interval) at 1 Hz, recorded in a vehicle-injected rat. First and 10th pair of responses are shown. The population spike evoked by the 1st stimulus increased greatly and remained potentiated for >10 min. At no point were multiple population spikes evoked by a stimulus. Similar results were obtained whether the interstimulus interval was 40 or 70 ms. Note that the recording site for this particular experiment was close to CA3, explaining the presence of a small antidromic population spike (→). As the recording electrode is moved toward CA3, an increasingly large antidromic spike is recorded. In area CA3 proper, the antidromic spike is much larger than the orthodromic response (Scharfman and Goodman 1998). *Bottom*: responses to stimulation in an AOAA-treated rat at similar sites to the control rat described earlier, except that the recording site was closer to CA1b than CA3 (hence the lack of antidromic spike). After a 1-Hz train of paired pulses, multiple population spikes were produced. The first population spike was larger in amplitude after the 1-Hz train (i.e., it potentiated), but this amplitude change reversed within 30 s. *Inset, far right*: inflection on the response at *bottom, left* (AOAA, →), is unlikely to be due to AOAA treatment because it was observed in control rats as well. Depth profiles through CA1 and the dentate gyrus indicated that this inflection could be due to evoked activity in the dentate gyrus that is volume conducted to CA1. A depth profile is shown of responses to a CA3 stimulus recorded in CA1 and the dentate gyrus. Numbers beside the traces are depths in micrometers relative to the pyramidal cell layer (PCL). A positivity and small population spike is evoked in the dentate gyrus by the stimulus. The positivity occurs at an early latency, similar to the latency of the inflection marked (→). The ability of CA3 stimulation to activate the dentate gyrus was apparent only in more posterior locations, so it was not observed in the recording from the control rat in this figure (*top*), which was made at a relatively anterior site.

Alterations in synaptic responses of entorhinal and hippocampal neurons in AOAA-lesioned rats

ENTORHINAL CORTEX. *Responses to stimulation.* Responses to stimulation that were recorded in the superficial layers were abnormal in many of the AOAA-treated rats, both in vitro and in vivo. The results indicate that the neurons in the medial entorhinal cortex were hyperexcitable. Indeed, when examined intracellularly in vitro, sustained depolarizations with multiple discharges occurred in response to a single stimulus.

The reason for epileptiform responses was not clarified in the present study but is likely to be related to seizures or cell loss in the entorhinal cortex. It seems safe to reason that neuronal activity is influenced by loss of layer III neurons and some changes in layer II neurons. Because intraentorhinal AOAA injections also cause some neuronal degeneration in the presubiculum (Du et al. 1998) and because there is a GABAergic input from the presubiculum to the entorhinal cortex (van Haeften et al. 1997), loss of this projection also could contribute to chronic hyperexcitability. However, the GABAergic presubicular cells are a relatively small subpopulation, and their projections are limited topographically. Furthermore, they project to both principal and nonprincipal cells so that their loss would not necessarily lead to disinhibition of entorhinal neurons.

Besides cell loss, AOAA-induced seizures may have caused chronic alteration in entorhinal excitability by changes in the expression of genes that control the function of neurotransmitter receptors, ion channels, and growth fac-

tors (Gall 1993; Lason and Przewlocki 1994; Saffen et al. 1988; Sperk et al. 1986; Tonder et al. 1994; Tsaur et al. 1992; Vezzani et al. 1996) or by influencing neuron-glia interactions (Keyser and Pellmar 1994; Mennerick and Zorumski 1994; Steward et al. 1991). The prominent gliosis that occurs in the entorhinal cortex after AOAA treatment (Du et al. 1998) supports the hypothesis that changes in glia could alter excitability in the region. In addition, cell loss in layer III could have triggered changes in the surviving neurons, such as sprouting of axons and synaptic reorganization (see following text).

In some AOAA-treated rats, some slices were hyperexcitable but others were not. These differences could be due to variability in cell loss within the entorhinal cortex. The slices that had small responses to stimulation, for example, simply may have had more cell loss and as a result not produced repetitive, robust, and prolonged responses to stimulation. However, Nissl-stained sections of those slices were not clearly different from others. Another contributing factor may be that each stimulation site activated pathways that were slightly different, depending on the exact site of the electrode and dorsal-ventral part of the entorhinal cortex that was used and whether there was synaptic reorganization. The fact that variability was less evident in vivo supports the idea that variability was, at least in part, due to the slice preparation.

Responses to pre- and parasubiculum stimulation. There was a reduction in responses to pre- and parasubiculum stimulation in AOAA-treated rats. The reduction in presubiculum responses could be due to the fact that layer III neurons are likely to produce these responses normally (Caballero-Bleda and Witter 1993; Eid et al. 1996; Köhler 1984; Van Groen and Wyss 1990), and the majority of these neurons are lost after AOAA treatment. In contrast, layer II neurons are substantially less damaged by AOAA (Du and Schwarcz 1992; Du et al. 1998), so another mechanism is likely to be responsible for the loss of the layer II response to parasubiculum stimulation. One possibility is that changes in parasubicular neurons, and perhaps also in presubicular neurons, occur as a result of AOAA treatment and that these changes alter normal effects on entorhinal neurons. This scenario may include sprouting of target-deprived entorhinal afferents into the largely intact layer II or onto surviving layer III neurons.

Effects in the hemisphere contralateral to AOAA injection. It was surprising that the hemisphere contralateral to the lesion was altered in AOAA-treated rats because anatomic changes in this hemisphere are not prominent after AOAA treatment (Du et al. 1998). There could be several reasons for the bilateral effects of AOAA. First, the seizures produced by AOAA shortly after injection may activate neurons in both hemispheres, and subsequent seizure-induced effects might follow. In other words, the initial period of seizures after an AOAA-injection might ''kindle'' the brain bilaterally, leading to long-lasting changes in excitability. However, the repetitive activity required for kindling is likely to be longer than the relatively brief period of behavioral seizures experienced by AOAA-treated rats. Second, simply because cell loss is very sparse contralaterally does not exclude that changes in neurotransmitters, receptors, and modulators of synaptic transmission occur there. Third, cell loss in one entorhinal cortex is likely to reduce the normal afferent input to the contralateral entorhinal cortex because entorhinal neurons have contralateral projections (Lopes da Silva et al. 1990). This might trigger alterations in the contralateral circuitry that bias those neurons to become hyperexcitable. Cell loss in the presubiculum, although apparently minor, could contribute to contralateral changes because stimulation of presubicular commissural afferents have pronounced effects on the contralateral entorhinal cortex (Bartesaghi et al. 1988). The GABAergic component of the presubiculum (discussed earlier) projects to the contralateral entorhinal cortex (Van Haeften et al. 1997), so loss of this pathway also could lead to contralateral changes.

HIPPOCAMPUS. *Dentate gyrus.* The lack of changes in perforant path input to granule cells both in vitro and in vivo is consistent with the fact that the AOAA lesion largely spares the layer II perforant path projection to the dentate gyrus. The fact that paired-pulse and frequency-dependent inhibition were unchanged is consistent with the dependence of this type of inhibition on dentate local circuit neurons, which are not lesioned by AOAA treatment. However, the alterations in spontaneous rhythms indicate that there were some effects of the lesion on dentate gyrus activity. There were two changes. One was a reduction in theta amplitude that was recorded in the outer molecular layer. This could reflect alterations in the input from layer II neurons that project to the dentate gyrus (because of the loss of the normal afferent input from layer III to layer II) (Köhler 1986a,b). However, there also was decreased theta in stratum lacunosum-moleculare; this raises the possibility that the changes recorded in the outer molecular layer were merely ''volume conducted'' from stratum lacunosum-moleculare, i.e., reflect reduced theta in stratum lacunosum-moleculare without a true change in the dentate gyrus. A change in theta in stratum lacunosum-moleculare could be explained by the loss of afferents from layer III to this layer.

The second change was a disruption of the fast oscillations in the granule cell layer/hilus. This could be explained by an altered layer II input to dentate nonprincipal cells (Kiss et al. 1996; Zipp et al. 1989), which often have dendrites in the molecular layer, receive perforant path input, and may contribute to fast oscillations (Bragin et al. 1995). However, inputs from the entorhinal cortex to the dentate gyrus other than those from layers II and III (Deller et al. 1996b), such as those from the deep layers (Köhler 1985), also may have been altered.

Area CA1. It was surprising that responses of CA1 pyramidal cells to Schaffer collateral stimulation in vitro were not different from controls, given the evidence from hilar stimulation that area CA3 neurons, the axons of which comprise the Schaffer collaterals, were hyperexcitable. Therefore, the hyperexcitability observed in CA3 may only be triggered by mossy fiber stimulation, indicating selectivity. The results therefore suggest that AOAA-induced lesions cause a selective change in the mossy fiber-evoked response of CA3 pyramidal cells rather than a universal change in afferent responses in hippocampus.

It was also surprising that responses to stratum lacunosum-moleculare stimulation in slices from AOAA-treated rats were not different from controls because layer III innervates

CA1 in this stratum, and layer III was substantially lesioned. The lack of differences may be due to the partial nature of the lesion and/or to compensation by surviving layer III cells. In fact, stimulation of stratum lacunosum-moleculare may not be an ideal index of layer III function because it also would activate the pathway from the nucleus reuniens (Dolleman-Van der Weel et al. 1997). Furthermore, innervation of CA1 by layer III neurons is complex in that both inhibitory neurons and pyramidal cells may be innervated (Buzsaki et al. 1995; Desmond et al. 1994; Doller and Weight 1982; Empson et al. 1995; Kiss et al. 1996; Leung 1995; Levy et al. 1995; Soltesz and Jones 1995; Yeckel and Berger 1995).

Area CA1, in vivo versus in vitro results. Although in vitro recordings did not demonstrate abnormalities in CA1, in vivo recordings did (Denslow et al. 1995). The lack of evidence in vitro could be due to the fact that stimulation in slices is not identical to stimulation in vivo and that slices contain only a portion of the CA3-CA1 circuitry that exists in vivo. It is also possible that stimulation in vivo activated the mossy fibers as well as Schaffer collaterals by current spread into stratum lucidum, which is likely given the large diameter of the stimulating electrode used in vivo relative to the one used in vitro ($\sim$500 vs. 75 μm diam). If mossy fibers were activated, it is likely that multiple CA3 population spikes would be evoked, and these would in turn lead to multiple CA1 spikes.

It is also possible that loss of the entorhinal excitatory input to interneurons in CA1 leads to disinhibition specifically of the commissural input. This could occur if these interneurons innervate the terminals of the commissural axons and hence inhibit release of glutamate under normal conditions; loss of excitatory input from the entorhinal cortex onto these interneurons then would disinhibit the terminals and could lead to increased glutamate release per stimulus. However, it is not known that interneurons in CA1 that receive input from the entorhinal cortex specifically innervate Schaffer collateral terminals. It is also not clear that any type of interneuron in CA1 specifically innervates nerve terminals, although some interneurons do target the axon hillock (Li et al. 1992). Moreover, such a circuit cannot completely explain the present results because such disinhibition would likely facilitate processes like potentiation (GABA antagonists facilitate long-term potentiation) (Mott and Lewis 1991; Wigstrom and Gustafsson 1983), whereas the duration of potentiation appeared to be impaired in CA1 of AOAA-treated rats (see following text).

Area CA3

Area CA3 neurons demonstrated multiple population spikes in response to repetitive stimulation of the mossy fiber input, and sometimes spreading depression, in AOAA-treated rats, indicating abnormally strong excitation by this input. Such a change could be related to loss of normal perforant path input, which normally innervates the region where their distal apical dendrites reside (Hjorth-Simonsen and Jeune 1972; Steward 1976; Steward and Scoville 1976; Wyss 1981). If this input normally excites interneurons, which in turn innervate pyramidal cells, the loss of the interneurons might leave area CA3 pyramidal cells disinhibited. On the other hand, there is strong evidence that the perforant path excites CA3 pyramidal cells directly (Wu and Leung 1998; Yeckel and Berger 1995), although it must be noted that most of this projection arises from entorhinal layer II neurons, which are not substantially damaged by AOAA. Thus there also may be changes in the intrinsic properties of CA3 neurons of AOAA-treated rats (perhaps in reaction to the loss of perforant path input to their dendrites) or changes in the mossy fiber axons of granule cells. Changes in granule cells could have been triggered by the seizures after AOAA injection, which is particularly likely given that seizures can produce numerous changes in granule cell gene expression (Gall 1993; Marksteiner et al. 1990; Morgan et al. 1987; Saffen et al. 1988; Tonder et al. 1994; Tsaur et al. 1992). Changes in CA3 responses to mossy fiber stimulation also may be due to indirect mechanisms. For example, a loss of inhibitory input from the entorhinal cortex to hilar neurons could disinhibit CA3 neurons because inhibitory hilar neurons are thought to inhibit CA3 pyramidal cells (Bragdin et al. 1995; Müller and Misgeld 1990).

Implications

SYNAPTIC REORGANIZATION. Some of the in vitro data suggest that synaptic reorganization in the entorhinal cortex may have occurred in AOAA-treated rats. Thus some deep layer neurons in slices of AOAA-treated rats exhibited excitatory activity at long latencies after the stimulus. This type of activity is not due to disinhibition because it is not caused by GABA-receptor blockade (Jones and Lambert 1990a,b). It also is unlike the hyperexcitability produced by the muscarinic agonist carbachol (Dickson and Alonso 1997). Synaptic reorganization also was indicated by the CA1 responses to stratum lacunosum-moleculare stimulation; the responses were robust in spite of the loss of many ipsilateral layer III neurons. We are currently examining whether the chronological development of AOAA-induced hyperexcitability parallels the gradual emergence of such rearranged synaptic contacts.

We speculate that the stimulus for the reorganized circuits, if they are indeed formed, was cell loss after AOAA-induced seizures. Cell loss can trigger reorganization, particularly among those surviving neurons that had innervated the neurons that died. For example, axons of dentate granule cells reorganize after their target hilar neurons die (Cronin and Dudek 1988; Sutula et al. 1988; Tauck and Nadler 1985). In the medial entorhinal cortex, loss of layer III neurons (normal targets of deep layer neurons) (Köhler 1986a,b) could trigger sprouting of layer V/VI axons onto residual neurons in layer III or onto other neurons located close by. One possibility that would be consistent with the ensuing hyperexcitability would be sprouting onto apical dendrites of deep layer pyramidal cells. This would increase excitatory input to those neurons and potentially form recurrent excitatory circuits. Moreover, neurons in layers V/VI might make new synapses with basal dendrites of layer II neurons that normally are located in layer III, or somata of layer II neurons that are displaced into layer III after AOAA-induced lesions. All these changes would lead to enhancement of the normal excitatory pathway from deep layer neurons to layer II neurons. Another example of reorganization that might

have similar consequences would involve sprouting of presubicular fibers, which have lost their normal layer III targets, onto layer II neurons. None of these circuits are mutually exclusive, and complex reorganized circuits may result.

Notably, the results of this study do not suggest that substantial synaptic reorganization occurred in the hippocampus as has been described after large entorhinal lesions. Studies of complete entorhinal ablation demonstrated several changes in the dentate gyrus, such as dramatic alterations in the molecular layer (Caceres and Steward 1983; Deller et al. 1996a; Matthews et al. 1976a,b; Steward and Vinsant 1983). Physiological changes also occur (Clusmann et al. 1994; Reeves and Steward 1988; West et al. 1975). In AOAA-treated animals, there was no evidence of these changes.

COGNITIVE FUNCTION. One of the results of the present study was that CA1 neurons in AOAA-treated rats lacked lasting potentiation in response to repetitive stimulation of the contralateral CA3 region. This suggests a link between seemingly disparate, previous findings: that potentiation may be a substrate for learning and memory (Barnes 1995; Dudai 1989; Lisman 1994), that the entorhinal cortex plays an important role in learning and memory (Zola-Morgan et al. 1986), and that temporal lobe epileptics (which often sustain cell loss in the entorhinal cortex; see INTRODUCTION) usually have deficits in learning and memory (Thompson 1991). The results of this study suggest how these observations may be related. In other words, learning and memory deficits in individuals suffering from temporal lobe epilepsy may arise from alterations in the ability of limbic circuits to sustain potentiation.

EPILEPTOGENESIS. The results support the hypothesis that unilateral damage confined to the parahippocampal region, and specifically to the superficial layers of the entorhinal cortex, leads to lasting changes in excitability of parts of the entorhinal cortex and hippocampus. The damage, by itself, does not appear to lead to chronic behavioral (motor) seizures, but spontaneous seizures may be detectable by continuous EEG monitoring, which is currently being performed in our laboratories. Furthermore, the lesion may lead to seizure activity of limited scope, and predispose the parahippocampal region for seizures after another insult (mechanical, stress-related, etc.) to the area. The results suggest that damage to layer III of the entorhinal cortex is proconvulsant. However, the results do not prove that entorhinal cell loss alone (i.e., without seizures) can cause hyperexcitability because the treatment that was used produced seizures as well as cell loss. Further studies will be required to discriminate between the role of seizures and the role of cell loss in epileptogenesis.

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