Acta Medica Okayama

Volume 46, *Issue* 2 1992 *Article* 10

APRIL 1992

Mechanisms in the development of limbic status epilepticus and hippocampal neuron loss: an experimental study in a model of status epilepticus induced by kindling-like electrical stimulation of the deep prepyriform cortex in rats.

Koutaro Inoue* Kiyoshi Morimoto† Keiko Sato‡
Norihito Yamada** Saburo Otsuki††

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
††Okayama University,

Mechanisms in the development of limbic status epilepticus and hippocampal neuron loss: an experimental study in a model of status epilepticus induced by kindling-like electrical stimulation of the deep prepyriform cortex in rats.*

Koutaro Inoue, Kiyoshi Morimoto, Keiko Sato, Norihito Yamada, and Saburo Otsuki

Abstract

A new model of status epilepticus (SE), which was induced by intermittent electrical stimulation (20 Hz for 20 sec every min for 180 min) of the deep prepyriform cortex, has been developed in the conscious rat. SE was induced in 9 of 16 rats in the drug-free group. The number of stimulation trains required to induce SE in this status subgroup was 125.6 +/- 12.7 (mean +/- SEM) and the mean duration of self-sustained seizure activity (SSSA) occurring after cessation of the stimulation session was 295.4 +/- 111.4 min. Some animals showed secondary generalized seizures. Significant cell loss was observed in the hippocampal CA3 pyramidal cell layer ipsilateral to the stimulation site and bilateral CA1 areas in the status subgroup compared with the group subjected to sham operation. In addition, there was a significant negative correlation between the duration of SSSA subsequent to the stimulation session and the total number of intact pyramidal neurons observed in the bilateral CA1 and ipsilateral CA3 subfields of the status subgroup. There were significant differences between the status and non-status subgroups with respect to the number of afterdischarges (ADs) and the total AD duration during the stimulation session. Pretreatment with phenobarbital (30 mg/kg) prevented the development of SE and hippocampal cell loss completely. Pretreatment with MK-801, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (0.25 or 1 mg/kg), also prevented hippocampal cell loss, although it did not block SE generation completely, which suggests dissociation of the mechanisms underlying the development of SE and hippocampal damage. These results indicate that prolonged SSSA actually causes hippocampal damage and it is critically dependent upon NMDA receptor participation.

KEYWORDS: status epilepticus, deep prepyriform cortex, electrical stimulation, hippcampus, N-methl-D-aspartate(NMDA), ??-aminobutyric acid(GABA)

*PMID: 1533485 [PubMed - indexed for MEDLINE]

Acta Medica Okayama 46 (2) 129-139 (1992)

Mechanisms in the Development of Limbic Status Epilepticus and Hippocampal Neuron Loss: An Experimental Study in a Model of Status Epilepticus Induced by Kindling-Like Electrical Stimulation of the Deep Prepyriform Cortex in Rats

Koutaro Inoue*, Kiyoshi Morimoto, Keiko Sato, Norihito Yamada and Saburo Otsuki

Department of Neuropsychiatry, Okayama University Medical School, Okayama 700, Japan

A new model of status epilepticus (SE), which was induced by intermittent electrical stimulation (20 Hz for 20 sec every min for 180 min) of the deep prepyriform cortex, has been developed in the conscious rat. SE was induced in 9 of 16 rats in the drug-free group. The number of stimulation trains required to induce SE in this status subgroup was 125.6 ± 12.7 (mean ± SEM) and the mean duration of self-sustained seizure activity (SSSA) occurring after cessation of the stimulation session was 295.4 ± 111.4 min. Some animals showed secondary generalized seizures. Significant cell loss was observed in the hippocampal CA3 pyramidal cell layer ipsilateral to the stimulation site and bilateral CA1 areas in the status subgroup compared with the group subjected to sham operation. In addition, there was a significant negative correlation between the duration of SSSA subsequent to the stimulation session and the total number of intact pyramidal neurons observed in the bilateral CA1 and ipsilateral CA3 subfields of the status subgroup. There were significant differences between the status and non-status subgroups with respect to the number of afterdischarges (ADs) and the total AD duration during the stimulation session. Pretreatment with phenobarbital (30 mg/kg) prevented the development of SE and hippocampal cell loss completely. Pretreatment with MK-801, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (0.25 or 1 mg/kg), also prevented hippocampal cell loss, although it did not block SE generation completely, which suggests dissociation of the mechanisms underlying the development of SE and hippocampal damage. These results indicate that prolonged SSSA actually causes hippocampal damage and it is critically dependent upon NMDA receptor participation.

Key words : status epilepticus, deep prepyriform cortex, electrical stimulation, hippocampus, N-methyl-D-aspartate (NMDA), γ-aminobutyric acid (GABA)

It has been well documented that the hippocampus is a particularly susceptible site for brain damage in epileptic patients. Over 100 years ago, Sommer (1) provided the first histological description of hippocampal damage, and several subsequent studies have yielded further information about patterns of hippocampal cell loss in patients with epilepsy (2, 3). The association of seizures and temporal lobe epilepsy with the lesion of hippocampal sclerosis has been

* To whom correspondence shold be addressed.

recognized for many years (4, 5), but there has been considerable debate as to whether hippocampal sclerosis is a cause or an effect of seizures. In one study, the severity of neuronal loss appeared to be related to the duration of the seizure disorder and to the frequency of generalized tonic-clonic seizures (3), a finding which supports the view that the lesion is an effect of seizures. However, other quantitative studies have failed to establish a clear relationship between neuronal loss and seizure history (2).

As human material can provide only limited information about the pathogenesis of brain changes associated with epilepsy, several attempts have been made to create animal models of status epilepticus (SE) with self-sustained seizure activity (SSSA).

The SE models used most often are produced by administering convulsive agents, such as kainic acid (KA) (6), glutamic acid (7), bicuculline (8) and lithium-pilocarpine (9) to experimental animals. However, these models present several interpretive problems. It is difficult to exclude direct neurotoxic effects of the convulsant *per se* on the central nervous system, and determination of its regional distribution may be difficult. Also, the neuronal pathways involved in the initiation and propagation of the seizure cannot be specified precisely. Some of these interpretive problems can be circumvented by models of SE induced by electrical stimulation. However, only a few studies using electrical stimulation-induced SE model have been reported (10–14).

We have been investigating the excitatory role of N-methyl-D-aspartate (NMDA) receptors and the inhibitory role of γ-aminobutyric acid (GABA) receptors in the kindling model of epilepsy (15–18). Our results indicate that the collapse of GABA-mediated inhibition and subseqent activation of NMDA receptors are essential for kindling. It also has been hypothesized that a loss of GABA-containing cells (19) or a functional loss of the hippocampal GABAergic inhibitory system (13, 20) causes an excitatory-inhibitory imbalance, which leads to seizure activity.

Recent experimental evidence suggests that overactivation of NMDA receptors contributes to the process underlying the hippocampal damage observed in epilepsy (21–24) and other pathological conditions, such as ischemia-hypoxia (25–28), hypoglycemia (29) and mechanical trauma (30). Therefore, NMDA receptor antagonists, which are potent antiepileptogenic agents, may be expected to block both SE and seizure-related hippocampal damage. However, the relationship between SE and hippocampal neuronal damage is still unclear, because some recent noteworthy studies have shown that the neuroprotective effect of NMDA receptor antagonists is not always associated with an antiepileptic effect (21, 22, 31).

In this study, we have developed a new rat model of SE, in which the deep prepyriform cortex (DPC) was stimulated electrically, in a manner similar to that which induces kindling. The DPC has been shown to be one of the most crucial epileptogenic sites by Piredda and Gale (32). Furthermore, in order to investigate the mechanisms underlying SE and hippocampal damage, we have studied the effects of a non-competitive NMDA receptor antagonist, (+)-5-methyl-10, 11-dihydro-5H-dibenzo (a, d) cyclohepten-5, 10-imine maleate (MK-801), and a $GABA_A$ receptor effector, phenobarbital (PB), in this model.

Materials and Methods

Forty-four male Sprague-Dawley rats, weighing 280–340 g at the time of surgery, were used. They were housed in a temperature-controlled colony with a 12 h light-12 h dark cycle and allowed free access to food and water. They were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and tripolar stimulation-recording electrodes, fashioned from twisted (0.18 mm diameter) Diamel-insulated Nichrome wire, were implanted stereotaxically into the left DPC (4.0 mm anterior and 3.3 mm lateral to bregma and 6.8 mm below the dura with the incisor bar 5.0 mm above the interaural plane). A screw electrode placed on the right frontal skull served as a

recording indifference. The electrode leads were mounted in a miniature connecotor and fixed to the skull with dental acrylic.

After a postoperative recovery period of at least 7 days, the rats were divided into 4 groups (drug-free, n=16; MK, n=12; PB, n=7; sham, n=9). The drug-free rats were placed in an electriacally screened recording chamber and the electrodes were connected to an amplifier (Nihonkoden AB-621G), an EEG polygraph (Nihonkoden WI-621GS) and a microcomputer-controlled stimulator system (microcomputer: Fujitsu FM16B-FD1; stimulator: Physio-Tech DS-503; isolator: Physio-Tech IS-500B). The DPC of each rat was stimulated by a train of biphasic square pulses at 20 Hz, with a pulse duration of 250 μsec and pulse intensity of 200 μA, for 20 sec every minute for 180 min via two poles of the tripolar electrode. The EEG was recorded from the remaining pole and skull screw electrode both during and after cessation of the electrical stimulation period. For quantitative analyses, we measured both the total duration of seizure activity during the period of stimulation and the duration of continuous seizure activity occurring after the stimulation session had finished. These measurements only included the periods of synchronized spikes or spike-wave complexes with amplitudes that exceeded twice that of the baseline EEG, which demonstrated frequencies greater than 1 Hz. When uninterrupted seizure activity persisted for more than 30 min we considered that the animal had developed SE. The behavioral seizure manifestation also was assessed, using a modified Racine's classification (33), as follows: stage 0, no response or behavioral arrest; stage 1, rhythmic mouth and facial movements; stage 2, rhythmic head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus with rearing; stage 5, rearing and falling. The MK and PB group rats received (i.p.) an NMDA receptor antagonist, MK-801 (0.25 mg/kg, n=6; 1 mg/kg, n=6) and a $GABA_A$ receptor effector PB (30 mg/kg, n=7) respectively 2 h prior to being subjected to the stimulation protocol, as described for the drug-free group. The sham group rats were subjected to sham operation only and did not undergo electrical stimulation.

Seven days after the SE-induction experiments, the animals were anesthetized with an overdose of pentobarbital and perfused transcardially with 0.9 % (w/v) saline followed by 10 % (w/v) formalin. The formalin-fixed brains were cut into blocks, which comprised the rostral (including the DPC) or caudal (including the hippocampus) parts, and paraffin embedding was performed. In order to verify the electrode positions, serial coronal slices 8 μm-thick were cut from the blocks every 160 μm and stained with hematoxylin-eosin. For histopathological examination of the hippocampus, 8 μm-thick sections, including the dorsal hippocampus, which was located 2.4–2.8 mm posterior to the bregma (34), were prepared on a sliding microtome and stained with cresyl violet. Three sections, 40–80 μm apart, from each animal were used for cell counting. The number of pyramidal neurons was counted by one of us, who, at the time of counting, was unaware of the experimental manipulation. The number of intact neurons per 250 μm linear length of the stratum pyramidale within the CA1 or CA3 subfields on each side was counted at a magnification of $\times$400; using a microscope (Nikon LURE 1). Neurons which had shrunken cell bodies with surrounding empty spaces were excluded. Three different sections from each rat were examined and the average of the three values obtained was taken as the neuronal density.

All data obtained were expressed as means $\pm$ SEM. Statistical analyses were performed using the Mann-Whitney's U-test to compare the electrobehavioral and histological parameters and Fisher's exact probability test to compare the differences between the treatment groups. Differences at p values of less than 0.05 were considered to be significant. The correlation between the hippocampal cell loss and SSSA duration was calculated using simple regression analysis.

Results

Electrical stimulation of the DPC induced SE in 9 of 16 conscious freely moving rats in the drug-free group, which we divided into two subgroups; one consisting of rats in which SE was induced (status subgroup: n=9) and the other of those in which it was not (non-status subgroup: n=7). Animals from the status subgroup typically showed stimulus-induced afterdischarges (ADs) (Figs. 1 A and B) that eventually exceeded the intertrain interval (Fig. 1 C) and ultimately resulted in prolonged SSSA (Figs. 1 D and E). They also showed various stages of motor seizures, the type of which are usually observed during kindling. After cessation of stimulation, SSSA continued for some time (90–1140 min), during which intermittent limbic seizures occurred (Fig.

1 D).

For the status subgroup, the mean number of trains required to produce SE was 125.6 ± 12.7, the mean duration of SSSA subsequent to the stimulation session was 295.4 ± 111.4 min, and post-stimulation secondary generalized seizures occurred in 3 of the 9 rats.

Quantitative analysis of the electrographic data revealed significant differences between the status and non-status subgroups with respect to both the number of ADs ($p < 0.01$) and the total AD duration ($p < 0.01$) during the stimulation session (Table 1). However, the number of generalized seizures occurring during the stimulation session in the two subgroups did not differ significantly (Table 1).

We examined the distribution of the electrode tips, but there was no systematic difference between the status and the non-status subgroups (Fig. 2).

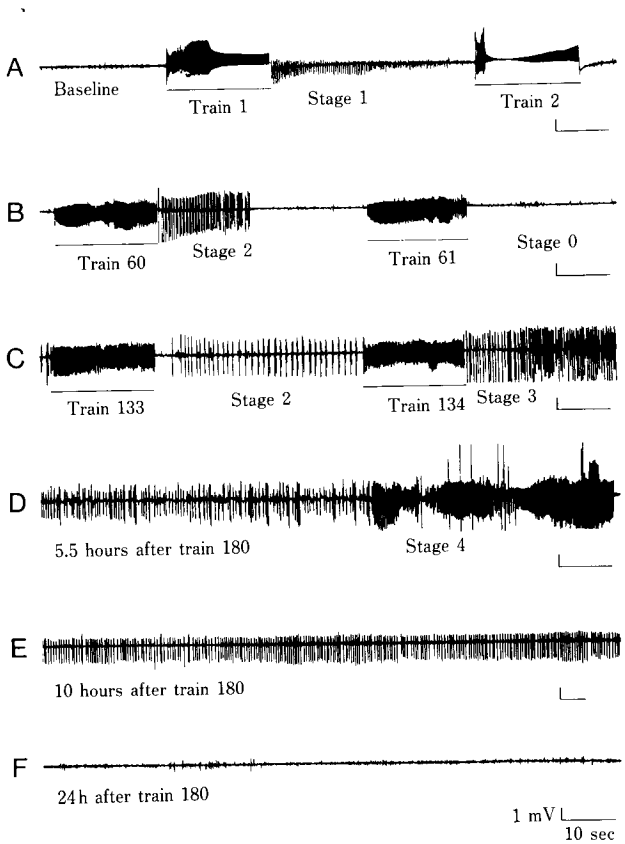

Fig. 1 An example of an EEG recorded from the DPC of a rat in the status subgroup at various times during and after the stimulation session. The DPC was stimulated at 20 Hz, for 20 sec, at intervals of 40 sec (indicated by underlining and the train numbers). The first stimulus train elicited an AD and a stage 1 behavioral seizure (A). Although kindling-like progress of behavioral seizures was observed, some trains failed to induce an AD (B). Further stimulation eventually led to an AD that outlasted the stimulation interval (C), at which point the seizune activity became self-sustaining. After cessation of stimulation, this seizune activity, which satisfied the criterion of exceeding twice the baseline EEG amplitude and a frquency in excess of 1 Hz, continued uninterrupted for 10 h (E). In addition, a spontaneous secondary generalized seizure associated with high frequency discharge occurred (D). The EEG recorded 24 h after train 180 did not differ markedly from the baseline EEG (F).

Table 1 Electrobehavioral parameters of the rats from the status and non-status subgroups

	Number of generalized seizures	Number of afterdischarges	Total afterdischarge duration (sec)
Status	31.6 ± 13.9	140.7 ± 6.7**	3565.0 ± 382.5**
Non-status	11.0 ± 2.9	84.6 ± 9.3	1246.7 ± 144.5

Status: n=9, Non-status: n=7, Value: mean ± SEM, ** $p < 0.01$, Mann-Whitney's U test compared with non-status subgroup

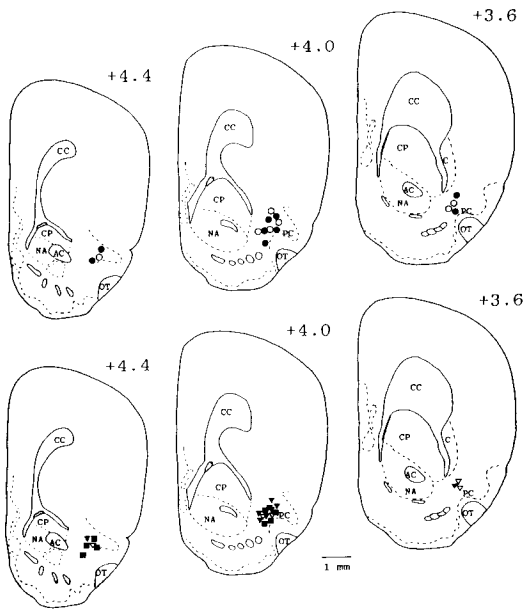

Fig. 2 Loci of the electrode tip centers estimated by histological examination. This diagram was produced, according to the Atlas of Pellegrino and Cushman (34). The numbers indicate the rostrocaudal levels of the sections in mm anterior to bregma. All the electrodes were located within the targeted sites. ●, status subgroup; ○, non-status subgroup; ■, sham group; ▽, MK group; ▼, PB group. AC, anterior commissure; C, claustrum; CC, corpus callosum; CP, caudate-putamen; NA, nucleus accumbens; OT, lateral olfactory tract; PC, pyriform cortex.

Histological study of the hippocampus revealed that the status subgroup (an example is illustrated in Fig. 4) underwent significant cell loss compared with the sham group ($p < 0.01$, Mann-Whitney's U-test) (Fig. 3, Table 2). Interestingly, the cell loss occurred in the hippocampal CA3 pyramidal cell layer ipsilateral to the stimulation site, whereas bilateral cell loss occurred in the CA1 subfields. No significant neuronal damage was observed in the CA3 subfield contralateral to the stimulation site (Table 2). In addition, further statistical analysis revealed significant differences of neuronal cell density between the right and left sides of the hippocampal CA1 and CA3 subfields (Table 2). Greater damage was observed in the left (ipsilateral to the stimulation site) than the right sides in both the CA1 and CA3 subfields. The non-status subgroup (n=7) showed no significant cell density changes of either CA1 or CA3 neurons compared with the sham group (Table 2).

For the status subgroup (n=9), a significant negative correlation was found between the duration SSSA subsequent to the stimulation session and the total number of intact pyramidal neurons remaining in the bilateral CA1 and ipsilateral CA3 subfields ($r = -0.834$; $p < 0.01$, n=9).

Only 1 of the 6 and 2 of the 6 animals pretreated with MK-801 (1 and 0.25 mg/kg), respectively and none of those from the PB group (n=7) developed SE, whereas 9 of the 16 animals in the drug-free group did, as described above (Table 3). Fisher's exact probability test revealed that the establishment of SE was prevented significantly by pretreatment with PB (30 mg/kg, $p = 0.0140$), but not by pretreatment with MK-801 (0.25 mg/kg, $p = 0.3176$; 1 mg/kg, $p = 0.1185$).

In the PB group, the mean number of ADs during the stimulation session was 81.9 ± 14.3, which did not differ significantly from that of the non-status subgroup, the mean total AD duration during the stimulation session was 558.0 ± 112.0

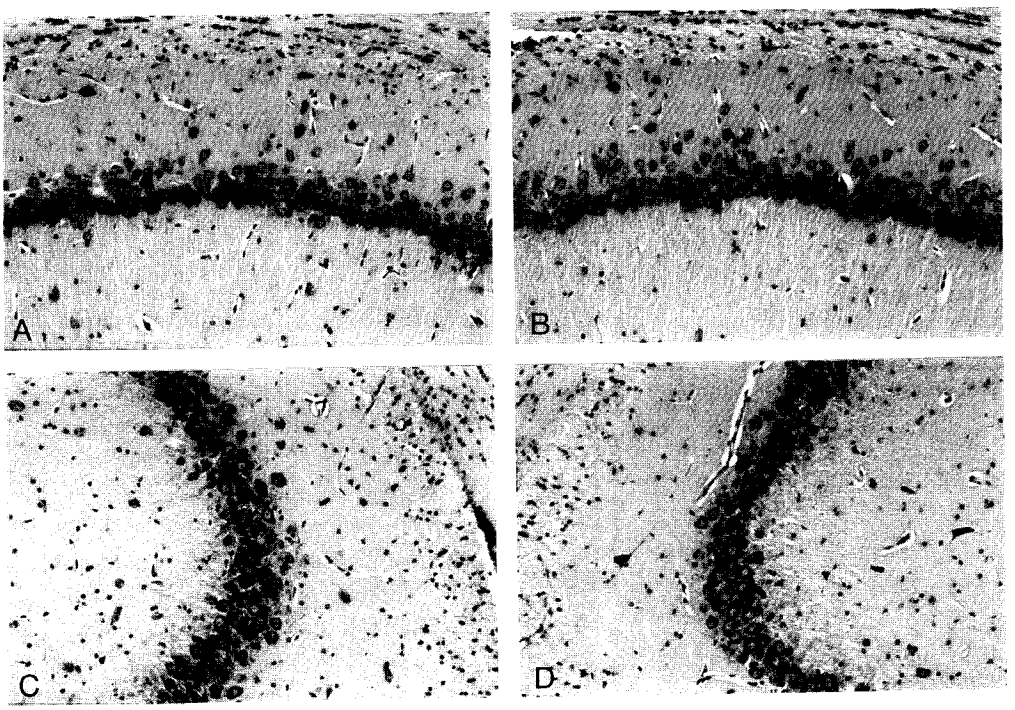

Fig. 3

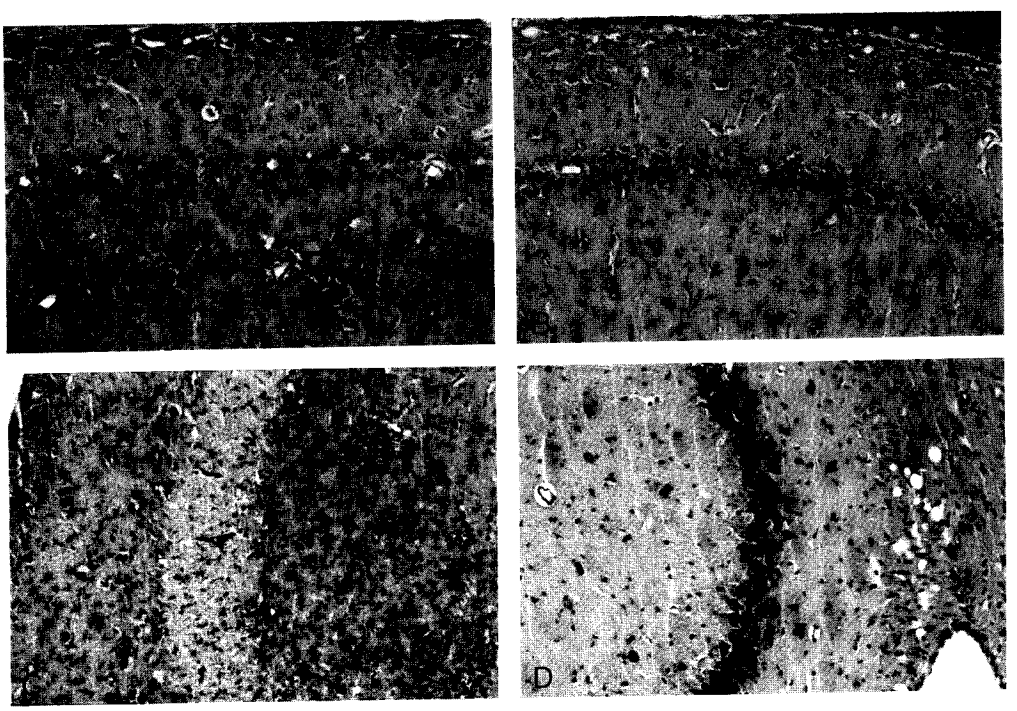

Fig. 4

Table 2 Neuronal cell density per 250 μm linear length of the CA1 and CA3 subfields

	n	CA1		CA3	
		Left	Right	Left	Right
Sham	9	47.4 ± 0.4	46.9 ± 0.7	28.3 ± 0.7	27.9 ± 1.2
Drug-free (status)	9	18.8 ± 3.1$^{\blacktriangledown\blacktriangledown}_{**}$	29.6 ± 3.9**	21.7 ± 2.7$^{\blacktriangledown}_{**}$	24.9 ± 1.3
Drug-free (non-status)	7	44.9 ± 1.6	46.7 ± 1.1	29.1 ± 1.2	28.7 ± 0.8
MK-801 0.25 mg/kg (status)	2	46.0 ± 1.2	47.0 ± 0.5	28.5 ± 0.1	29.3 ± 0.9
MK-801 1 mg/kg (status)	1	47.7 ± 0.0	46.0 ± 0.0	31.3 ± 0.0	27.7 ± 0.0

The status subgroup (n=9) showed significant cell loss in the bilateral CA1 and left (ipsilateral to the stimulation site) CA3 subfields compared with the sham group (n=9). In the non-status subgroup (n=7) no significant cell loss was observed at any of the sites investigated. Value: mean ± SEM, $**$ $p < 0.01$, Mann-Whitney's U test compared with sham group, $\blacktriangledown\blacktriangledown$ $p < 0.01$, $\blacktriangledown$ $p < 0.05$, Wilcoxon test compared with the contralateral side.

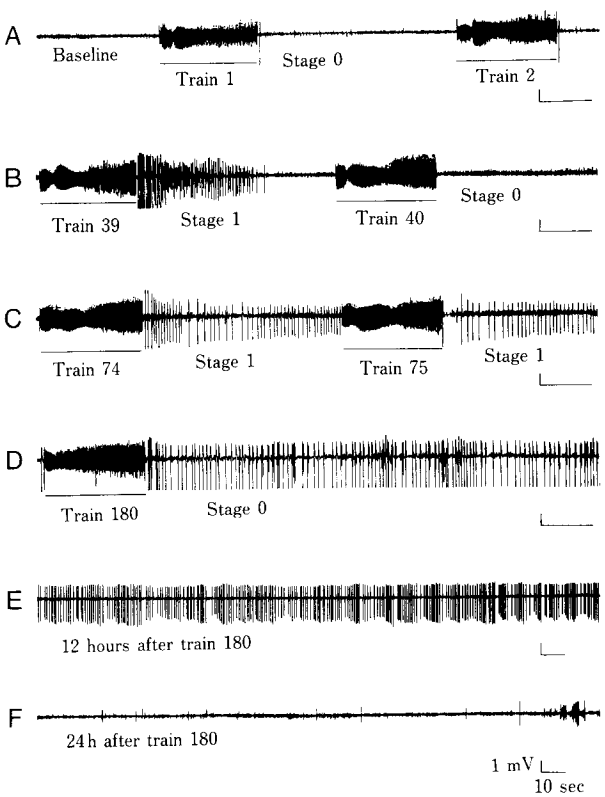

Fig. 5 An example of the EEG recorded from the left DPC of the rat pretreated with MK-801 (1 mg/kg) in which SE developed. In the early phase of the stimulation protocol, there was weak kindling-like progress of behavioral seizures (A, B). From train 74 onwards the seizure activity became continuous (C) and after train 180 the SSSA continued for about 12 h (D and E). Twenty-four hours after cessation of stimulation, the EEG was virtually the same as the baseline EEG (F).

Fig. 3 Coronal cresyl violet-stained sections of the dorsal hippocampal CA1 (A, B) and CA3 (C, D) subfields from a rat in the sham group. All photographs show the appearance of the pyramidal neurons to be normal. A, C: Left hippocampus. B, D: Right hippocampus. Magnification (A-D): × 150.

Fig. 4 Coronal cresyl violet-stained sections of the dorsal hippocampal CA1 (A, B) and CA3 (C, D) subfields from a rat in the drug-free group. This rat experienced status epilepticus for 1140 min after the cessation of stimulation. Severe cell loss was observed in the bilateral CA1 and left CA3 (C) subfields. A, C: Left hippocampus. B, D: Right hippocampus. Magnification (A-D): × 150.

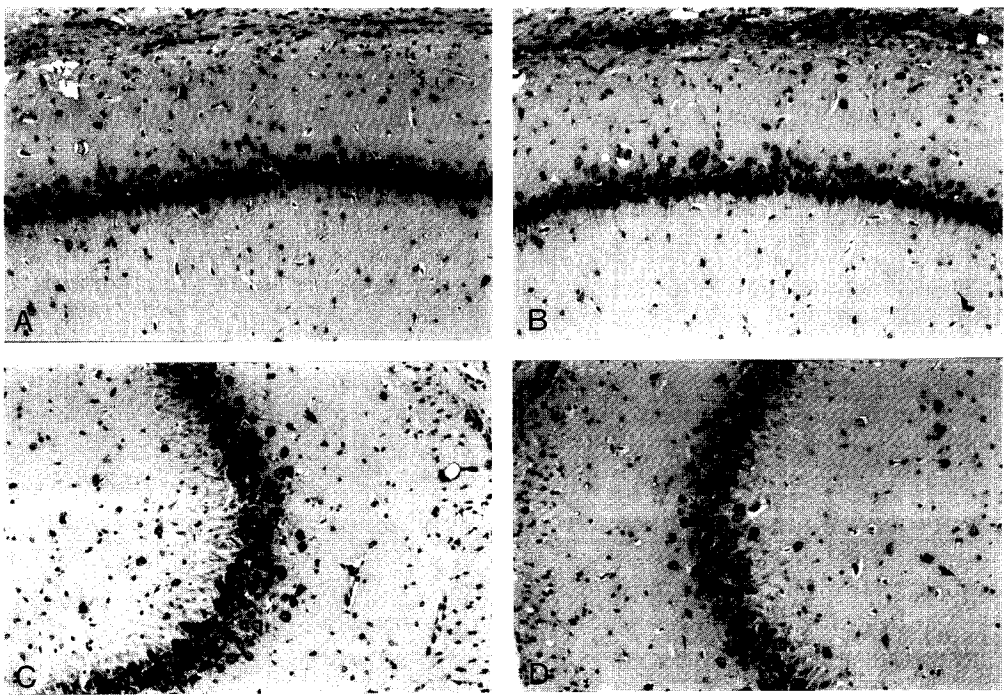

Fig. 6 Coronal cresyl violet-stained sections of the dorsal hippocampal CA1 (A, B) and CA3 (C, D) subfields from the same rat in the MK (1 mg/kg) group, for which the EEG is shown in Fig. 5. Although this rat experienced status epilepticus for 720 min after cessation of stimulation, no obvious pyramidal cell loss occurred. A, C: Left hippocampus. B, D: Right hippocampus. Magnification (A–D): × 150.

Table 3 The numbers of animals with SE (status) and without SE (non-status) in the drug-free, MK-801 (0.25 or 1 mg/kg) and PB (30 mg/kg) groups

		Status	Non-status
Drug-free		9	7
MK-801	0.25 mg/kg	2	4
	1.0 mg/kg	1	5
Phenobarbital	30 mg/kg	0*	7

* $p < 0.05$, Fisher's exact probability test compared with drug-free group.

sec which was significantly shorter than that of the non-status subgroup ($p < 0.01$), and no generalized seizures were observed.

One animal pretreated with MK-801 (1 mg/kg) showed typical SE after the 74 th stimulation and SSSA subsequent to stimulation that continued for 720 min (Fig. 5), although, histological examination of the hippocampus from this rat revealed no apparent pyramidal cell loss (Fig. 6, Table 2). Two other rats pretreated with MK-801 (0.25 mg/kg) experienced SE for 227.0 ± 2.1 min after cessation of stimulation, but no obvious pyramidal cell loss occurred in these two rats (Table 2).

Discussion

Intermittent electrical stimulation of the DPC for 3 h resulted in SE in 9 of 16 conscious, freely moving rats. In this status subgroup (n = 9), significant pyramidal cell loss was observed in the bilateral CA1 and ipsilateral CA3 hippocampal subfields. This pattern of pyramidal cell loss agrees with ischemic brain damage, which is most

severe in the CA1 region of the hippocampus (25-28). In contrast, in rodents following administration of KA CA3 region is preferentially damaged (6). One possibility for these different pattern of brain damage would be a correlation between vulnerability and density of subtype of glutamate receptors. In the hippocampus, high affinity KA receptors are dense around CA3. In contrast, NMDA receptors are absent in CA3 stratum lucidum whereas they are very dense in the CA1 strata oriens and radiatum (35).

Several recent studies have shown that direct electrical stimulation of hippocampal afferent pathways can produce limbic SE with hippocampal neuronal damage (13, 14, 23). In this study, it appeared that electrical stimulation to an other limbic structure (the DPC) which is distant from the hippocampus, can induce SE with hippocampal damage. In our preliminary experiments, SE was induced in 2 of 8 and 1 of 10 rats when the amygdala and the dorsal hippocampus respectively were subjected to the same stimulation protocol used in this study. Thus, the DPC tended to be more susceptible to electrical stimulation-induced SE than these sites. Because the DPC is fairly distant from the hippocampus, stimulation of it should have some advantage over other, nearer sites in that we could exclude the possibility of a direct effect of electrical stimulation of the hippocampus. Piredda and Gale (32) first reported that the DPC is extremely sensitive to chemoconvulsants, and there have been a few subsequent studies which demonstrated that the DPC is as highly susceptible to kindling as other limbic sites, such as the amygdala (36-38), and the DPC is believed to be one of the brain sites that can trigger electrically-induced SE with secondary generalized seizures.

In this study, we employed a stimulation protocol of 20 Hz, 20 sec trains at an intertrain interval of 40 sec. Vicedomini and Nadler (14) reported that the synaptic response of CA3 hippocampal neurons generated by perforant path stimulation was attenuated somewhat when the stimulus frequency was increased to above 20 Hz. They also observed that even tetanic stimulation for many hours failed to produce self-sustained seizures when the intertrain interval exceeded 90 sec. From these results, the stimulation protocol used in our study would appear to be for SE induction. Sloviter (13) noted that intermittent stimulation at 20 Hz to the perforant path caused an acute decrease in the GABAergic recurrent inhibition of dentate granule cells associated with seizure activity. This finding suggests that our stimulation protocol may be suitable for inactivating the GABAergic recurrent inhibitory system.

We have demonstrated that the duration of SSSA occurring after the cessation of stimulation correlated significantly with the magnitude of hippocampal cell loss. A similar correlation was found by Vicedomini and Nadler (14), who studied the model of SE induced by electrical stimulation of the hippocampal afferent pathways. Similarly, the magnitude and distribution of brain damage induced by intracerebroventricular administration of KA depended on the total seizure duration (39). Taken together, these results support the hypothesis that the duration of SSSA is the most critical factor in the pathogenesis of epileptic brain damage and that the brain damage is caused by SSSA. This hypothesis is consistent with the results of a clinical study in which the severity of neuronal loss appeared to be related to the duration of the seizure disorder and the frequency of generalized tonic-clonic seizures (3).

It is difficult to interpret the finding that 7 rats in the drug-free group subjected to electrical stimulation of the DPC did not develop SE. This result could not be attributed to differences in the distribution of the electrode tips (see Fig. 2). One possible explanation for this discrepancy is a heterogeneity in some intrinsic capability to counteract the recurrence of seizure activity (40). In fact, quantitative analysis revealed marked differences between the status and non-status subgroups with respect to the number of ADs and total AD duration during the stimulation session (Table 1). It is possible that some rats in

this non-status subgroup may have required more than 180 stimulations to develop SE, as Vicedomini and Nadler (14) reported that as many as 373 electrical stimulus trains to the perforant path or fimbria were required to induce SE.

All the rats pretreated with PB failed to develop SE and suffered no hippocampal damage, which agrees with the results of Ault *et al.* (39), who showed that pretreatment with 40 mg/kg PB prevented the development of KA-induced SE in 5 of 6 rats. These results indicate that failure of GABAergic inhibition is involved in the mechanism underlying the generation of SE. However, in the study of Ault *et al.* (39) one rat, which developed SE despite PB pretreatment, suffered brain damage, which was indistinguishable from that caused by KA alone. This finding suggests that, in contrast to its potent antiepileptogenic effect, this GABAergic agent may have a relatively weaker neuroprotective effect.

In our study, MK-801 also tended to inhibit the generation of SE, but this did not reach statistical significance. Surprisingly, one rat pretreated with MK-801 (1 mg/kg), which developed SE after 74 stimulation trains and experienced continuous SSSA for about 12 h demonstrated no hippocampal pyramidal cell loss (see Figs. 5 and 6). In addition, two rats pretreated with MK-801 (0.25 mg/kg), which experienced SE for about 3 h also demonstrated no hippocampal pyramidal cell loss (Table 2). The finding in these cases is in agreement with several recent studies using KA, which have demonstrated that the neuronal protective effect of this NMDA receptor antagonist was not necessarily accompanied by an antiepileptogenic effect (21, 22, 31). Therefore, the epileptic activity and neuronal damage may be subject to control by different mechanisms. In contrast, although MK-801 is considered to be an antiepileptogenic agent (16, 17), its failure to suppress the development of limbic SE raises questions about its potential as an antiepileptogenic agent for treating refractory seizure disorders, such as complex partial seizures or the SE syndrome. The NMDA receptor antagonists, however, could be used, perhaps, as neuroprotective agents in the interim period whilst seizure activity is being brought under control by other means, such as GABAergic agents.

In conclusion, the DPC is a brain site that can trigger electrically-induced SE with secondary generalized seizures and seizure-related hippocampal damage is caused by prolonged SSSA, which depends critically upon NMDA receptor participation.

Acknowledgments. This study was supported by the Japanese Epilepsy Research Foundation. The authors are grateful to Merck Sharp & Dohme Research Laboratories for supplying MK-801. We also thank Drs. H. Ishizu and K. Kawai for their help in improving the histological examination method and Mrs. M. Onbe for her excellent technical assistance.

References

1. Sommer W: Erkrankung des Ammonshorns als aetiologisches Moment der Epilepsien. Arch Psychiatr Nervenkr (1880) **10**, 631–675.
2. Babb TL, Brown WJ, Pretorius J, Davenport C, Lieb JP and Crandall PH: Temporal lobe volumetric cell densities in temporal lobe epilepsy. Epilepsia (1984) **25**, 729–740.
3. Dam AM: Epilepsy and neuron loss in the hippocampus. Epilepsia (1980) **21**, 617–629.
4. Sano K and Malamud N: Clinical significance of sclerosis of the cornu ammonis. Arch Neurol Psychiatr (1953) **70**, 40–53.
5. Stauder KH: Epilepsie und Schlafenlappen. Arch Psychiatr Nervenkr (1936) **104**, 181–211.
6. Ben-Ari Y: Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. Neuroscience (1985) **14**, 375–403.
7. Sloviter RS and Dempster DW: "Epileptic" brain damage is replicated qualitatively in the rat hippocampus by central injection of glutamate or aspartate but not by GABA or acetylcholine. Brain Res Bull (1985) **15**, 39–60.
8. Söderfeldt B, Kalimo H, Olsson Y and Siesjö B: Pathogenesis of brain lesions caused by experimental epilepsy: Light-and electron-microscopic changes in the rat cerebral cortex following bicuculline-induced status epilepticus. Acta Neuropathol (1981) **54**, 219–231.
9. Ormandy GC, Jope RS and Snead III OC: Anticonvulsant actions of MK-801 on the lithium-pilocarpine model of status epilepticus in rats. Exp Neurol (1989) **106**, 172–180.
10. Lothman EW, Bertram EH, Kapur J and Stringer JL: Recurrent spontaneous hippocampal seizures in the rat as a chronic sequela to limbic status epilepticus. Epilepsy Res

(1990) **6**, 110–118.

11. McIntyre DC, Nathanson D and Edson N: A new model of partial status epilepticus based on kindling. Brain Res (1982) **250**, 53–63.
12. Milgram NW, Green I, Liberman M, Riexinger K and Petit TL: Establishment of status epilepticus by limbic system stimulation in previously unstimulated rats. Exp Neurol (1985) **88**, 253–264.
13. Sloviter RS : "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path: I. Acute electrophysiological and light microscopic studies. Brain Res Bull (1983) **10**, 675–697.
14. Vicedomini JP and Nadler JV: A model of status epilepticus based on electrical stimulation of hippocampal afferent pathways. Exp Neurol (1987) **96**, 681–691.
15. Morimoto K: Seizure-triggering mechanisms in the kindling model of epilepsy: Collapse of GABA-mediated inhibition and activation of NMDA receptors. Neurosci Biobehav Rev (1989) **13**, 253–260.
16. Morimoto K, Katayama K, Inoue K and Sato K: Effects of competitive and non-competitive NMDA receptor antagonists on kindling and LTP. Pharmacol Biochem Behav (1991) **40**, 893–899.
17. Sato K, Morimoto K and Okamoto M: Anticonvulsant action of a non-competitive antagonist of NMDA receptors (MK-801) in the kindling model of epilepsy. Brain Res (1988) **463**, 12–20.
18. Sato K, Morimoto K, Okamoto M, Nakamura Y, Otsuki S and Sato M: An analysis of anticonvulsant actions of GABA agonists (progabide and baclofen) in the kindling model of epilepsy. Epilepsy Res (1990) **5**, 117–124.
19. Ribak CE, Bradburne RM and Basil-Harris A: A Preferential loss of GABAergic, symmetric synapses in epileptic foci: A quantitative structural analyses of monkey neocortex. J Neurosci (1982) **2**, 1725–1737.
20. Sloviter RS : Decreased hippocampal inhibition and selective loss of interneurons in experimental epilepsy. Science (1987) **235**, 73–76.
21. Clifford DB, Olney JW, Benz AM, Fuller TA and Zorumski CF: Ketamine, phencyclidine and MK-801 protect against kainic acid-induced seizure-related brain damage. Epilepsia (1990) **31**, 382–390.
22. Lerner-Natoli M, Rondouin G, Belaidi M, Baldy-Moulinier M and Kamenka JM: N-[1-(2-thienyl) cyclohexyl] - piperidine (TCP) does not block kainic acid-induced status epilepticus but reduces secondary hippocampal damage. Neurosci Lett (1991) **122**, 174–178.
23. Rogers BC, Barnes MI, Mitchell CL and Tilson HA: Functional deficits after sustained stimulation of the perforant path. Brain Res (1989) **493**, 41–50.
24. Ylinen A, Lahtinen H, Sirvio J, Partanen J, Asikainen A, Gulyas A, Freund TF and Riekkinen P: Behavioural, electrophysiological and histopathological changes following sustained stimulation of the perforant pathway input to the hippocampus: Effect of the NMDA receptor antagonist, CGP 39551. Brain Res (1991) **553**, 195–200.
25. Gill R, Foster AC and Woodruff GN: Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. J Neurosci (1987) **7**, 3343–3349.
26. Kato H, Araki T and Kogure K: Role of the excitotoxic mechanism in the development of neuronal damage following repeated brief cerebral ischemia in the gerbil: Protective effects of MK-801 and pentobarbital. Brain Res (1990) **516**, 175–179.
27. Rothman S: Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. J Neurosci (1984) **4**, 1884–1891.
28. Simon RP, Swan JH, Griffiths T and Meldrum BS: Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science (1984) **226**, 850–852.
29. Wieloch T: Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. Science (1985) **230**, 681–683.
30. Faden AI, Demediuk P, Panter SS and Vink R: The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science (1989) **244**, 798–800.
31. Fariello RG, Golden GT, Smith GG and Reyes PF: Potentiation of kainic acid epileptogenecity and sparing from neuronal damage by an NMDA receptor antagonist. Epilepsy Res (1989) **3**, 206–183.
32. Piredda S and Gale K: A crucial epileptogenic site in the deep prepiriform cortex. Nature (Lond) (1985) **317**, 623–625.
33. Racine RJ: Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroenceph Clin Neurophysiol (1972) **32**, 281–294.
34. Pellegrino LJ, Pellegrino AS and Cushman AJ: A Stereotaxic Atlas of the Rat Brain. Plenum Press, New York, 1979.
35. Cotman CW, Monaghan DT, Ottersen OP and Storm-Mathisen J: Anatomical organization of excitatory amino acid receptors and their pathways, Trends Neurosci (1987) **10**, 273–280.
36. Cain DP, Corcoran ME, Desborough KA and McKitrick DJ: Kindling in the deep prepyriform cortex of the rat. Exp Neurol (1988) **100**, 203–209.
37. Morimoto K, Dragunow M and Goddard G: Deep prepyriform cortex kindling and its relation to amygdala kindling in the rat. Exp Neurol (1986) **94**, 637–648.
38. Zhao DY and Moshé SL: Deep prepyriform cortex kindling and amygdala interactions. Epilepsy Res (1987) **1**, 94–101.
39. Ault B, Gruenthal M, Armstrong DR and Nadler JV: Efficacy of baclofen and phenobarbital against the kainic acid limbic seizure-brain damage syndrome. J Pharmacol Exp Ther (1986) **239**, 612–617.
40. Berger ML, Lassmann H and Hornykiewicz O: Limbic seizures without brain damage after injection of low doses of kainic acid into the amygdala of freely moving rats. Brain Res (1989) **489**, 261–272.

Received December 25, 1991 ; accepted January 31, 1992.