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Hippocampal plasticity in the kindling model of epilepsy in rats

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Histochemical and autoradiographic techniques were used to study mossy fiber sprouting which occurs in the hippocampus of kindled rats. In rats kindled by daily stimulation of amygdala or entorhinal cortex, the mossy fibers sprout to innervate the supragranular zone of fascia dentata, this synaptic reorganization was associated with a significant increase in the density of high-affinity kainic acid binding sites Amygdala but not entorhinal kindling also induces sprouting of mossy fibers in the stratum infrapyramidale of CA3

Kindling, an animal model of temporal lobe epilepsy [12], is associated with neuronal plasticity that may be relevant to the study of memory processes [16]. Because kindling is characterized by a persistent modification of synaptic responsiveness [6], numerous studies have been done to demonstrate changes in synaptic architecture [9] and fiber connections [15, 24]. In a recent report Sutula et al. [24] have shown that kindling induces, in the hippocampus, a sprouting of mossy fibers which establish aberrant connections upon the granular cells of fascia dentata Similar observations have been made after the administration of kainic acid (KA; an excitatory and neurotoxic dicarboxylic, analogue of glutamate [18], which reproduces the electrographic, behavioral and neuropathological sequelae of human temporal lobe epilepsy [1, 17]) High-affinity KA receptors are predominantly located in the hippocampus, on the mossy fiber terminals [21], a major input to pyramidal cells of CA3 originating in the granular cells of fascia dentata [8]. We have recently shown [21] that mossy fiber sprouting induced by KA treatment is associated with a significant increase in the density of KA binding sites in the reinnervated zones. This may promote further excitability of the hippocampal circuitry and contribute to epileptic disorders [5, 25].

In the present study we report that (1) kindling induces sprouting of hippocampal mossy fibers and an increase in KA binding sites in the abnormally innervated zones and (2) the mossy fiber sprouting differs according to the region stimulated; there is a particularly dense mossy fiber sprouting in the infrapyramidal region of CA3 following amygdala but not entorhinal kindling. Some of the results reported here have been published in abstract form [22].

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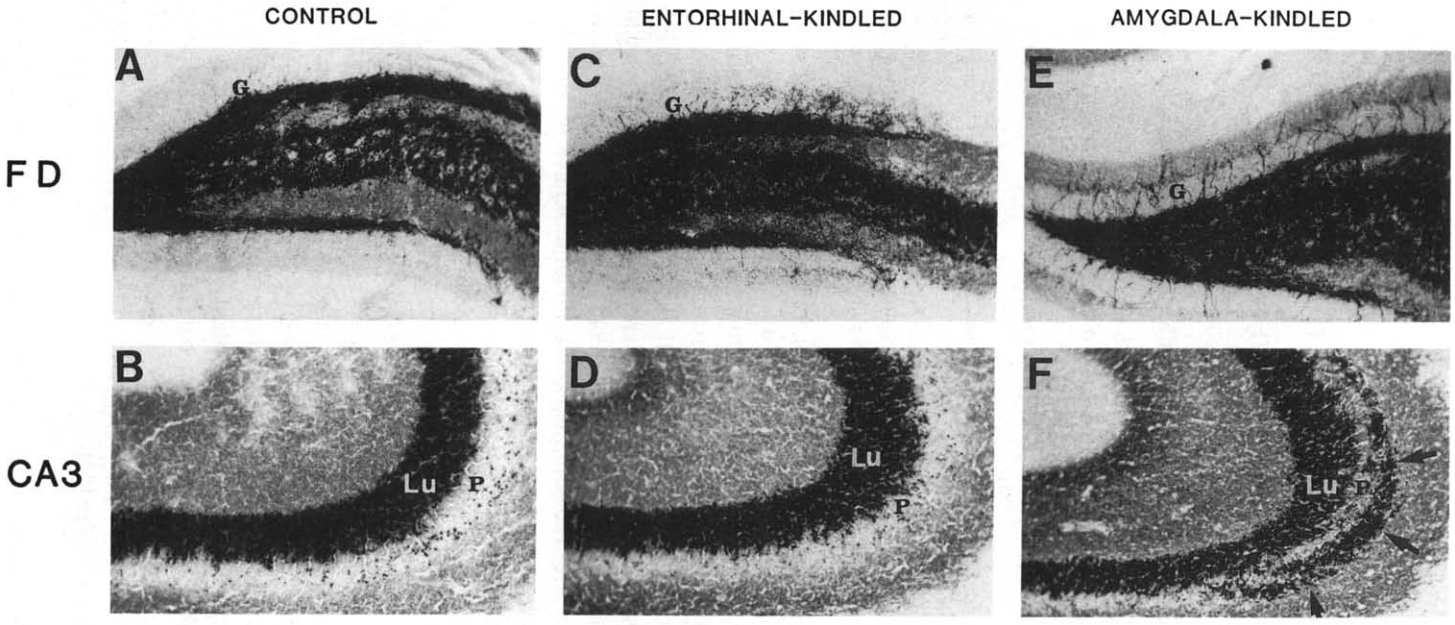

Fig 1 Effects of kindling on the pattern of Timm-stained mossy fibers in the rat hippocampus Note in the fascia dentata (FD, upper panel) the supragranular sprouting of mossy fibers in entorhinal- (C) and amygdala- (E) kindled rats In the CA3 region (lower panel) mossy fibers sprout and form an aberrant infrapyramidal band (arrows) of silver deposits, exclusively in the amygdala-kindled rats (F) p, stratum pyramidal, lu, stratum lucidum, G, granular layer

Adult Wistar rats of 220–250 g were used for these experiments. Under anesthesia with equithesin (4 ml/kg), stimulating-recording electrodes made of two stainless steel wires of 120 μm diameter were implanted into the amygdala or the entorhinal cortex, using conventional stereotaxic techniques. Two screws were also inserted into the skull, one for grounding the animal and another one for recording the sensory-motor cortex. Electrodes and screws were then soldered to a connector cemented on the skull One week after implantation, the afterdischarge threshold was determined for each animal using a 2 s train of 60 Hz square waves of 1 ms duration Stimulations were delivered twice a day until 3 consecutive stage 5 kindled seizures were elicited Amygdala and entorhinal kindling were achieved in respectively 11 and 27 stimulations

Under deep anaesthesia, control ($n=6$) and experimental ($n=10$) rats were perfused according to a slightly modified Timm's sulphide silver method [13] with phosphate buffer containing 0.14% sodium sulphide (200 ml), followed by 0 1% paraformaldehyde (200 ml) In a previous study [21] we found that this procedure (which selectively stains the mossy fibers) does not affect the K_d and B_{max} of KA binding. Coronal cryostat sections (20 μm) were cut throughout the hippocampus and used for binding and histological procedures in order to compare the distribution of mossy fiber terminals and KA binding sites in the same sections. The high-affinity KA binding sites with slow dissociation rate were visualized using a previously described autoradiographic technique [4]. In brief, after preincubation in Tris-acetate buffer (50 mM, pH = 7) to remove endogenous competitive ligand, the slices were incubated at 3°C in the same buffer containing 20 nM vinylidene-[^{3}H]KA (NEN, 60 Ci/mmol) in the presence (for non-specific binding) or absence (for total binding) of an excess of 10 μm cold KA. After rinsing for 2 min in 10 μm cold KA, to remove [^{3}H]KA from the readily dissociating low-affinity receptors [14], the sections were exposed to tritium-sensitive films concomitantly with plastic standards (Amersham [11]). For each case, at least 17 sections, from the septal to the temporal level of the hippocampus, were quantified with a computer-assisted image analyzer (IMSTAR) The statistical

TABLE I

MEAN DENSITIES (fmol/mg TISSUE ± S E M) OF [^{3}H]KA BINDING SITES IN KINDLED AND CONTROL HIPPOCAMPUS

	n	Fascia dentata (supragranular layer)	CA3		CA1
			Oriens	Lucidum	Radiatum
Control	6	19+2	13+2	59+4	16+2
Amygdala kindling	6	39+2*	51+4*	70+5*	20+7
Entorhinal C kindling	4	36+3*	17+6	54+3	18+4

*$P<0$ 001

analysis of data (in fmol/mg tissue) was performed with a Student's t-test. Alternate Nissl-stained sections confirmed the absence of histological lesions in kindled rats [24]

The Timm-stained slices obtained from entorhinal cortex (Fig 1C) and amygdala-kindled rats (Fig 1E) showed sprouting of mossy fibers in the fascia dentata, which crossed throughout the granular layer to form a clear supragranular band of silver deposits The quantitative study of KA binding sites (Table I) indicated that this sprouting was associated with a significant increase of the high-affinity KA binding sites in this zone (by 190%, $P<0.001$). In amygdala (Fig. 1F) but not in entorhinal (Fig. 1D) kindled rats, there was an abnormal sprouting of mossy fibers in the CA3 region As shown in Fig 1F, mossy fibers form a clear cut band of Timm deposits in the infrapyramidal layer of CA3 (the stratum oriens), which is associated with a striking rise (390%, $P<0.001$, Fig 2) in the density of KA binding sites (Fig 2C, also see Table I) Moreover in amygdala-kindled rats there was a small but significant rise (18%) in the density of KA binding sites in stratum lucidum All these effects extended bilaterally without obvious differences along the septo-temporal axis of the hippocampus except a slight predominance for the stimulated side.

The present results are in agreement with the recent observations of Sutula et al. [24] showing that kindling of limbic pathways induces a long-lasting synaptic reorga-

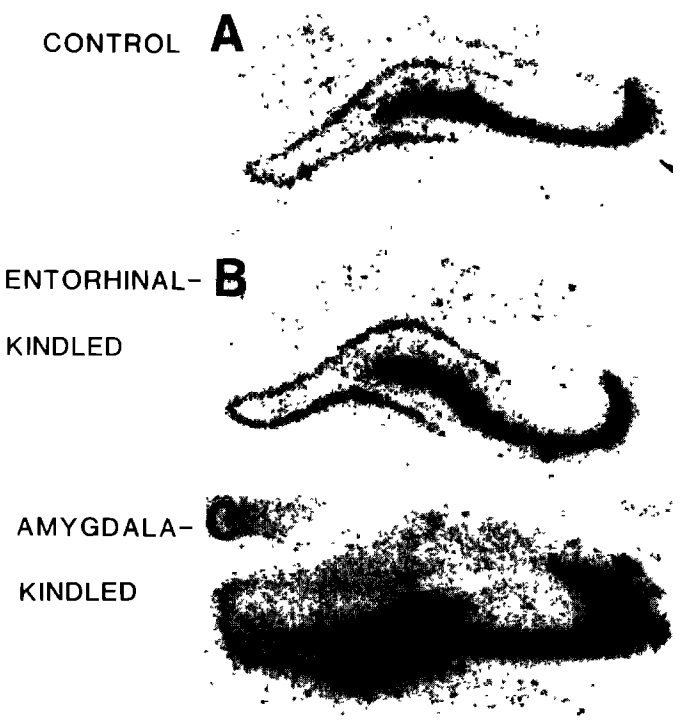

Fig 2 Photomicrographs depicting the distribution of [^{3}H]KA binding sites in a control (A) entorhinal- (B) and amygdala- (C) kindled rat Arrows show the aberrant infrapyramidal band of binding sites in kindled rats

nization of mossy fibers which sprout to abnormally innervate the granular cells of fascia dentata The increase in KA binding sites observed in kindled rats likely reflects the formation of new synaptic connections. In addition, we report that amygdala but not entorhinal kindling, also induces an abberant sprouting of mossy fibers in the infrapyramidal layer of CA3 This sprouting was not observed in the study of Sutula et al., since in the strain of rats they used (Sprague–Dawley) there is already an infrapyramidal bundle of mossy fibers

The sprouting of mossy fibers is associated with a significant increase in the density of high affinity KA binding sites. In contrast an earlier study [23] reported a loss of KA binding sites in the hippocampus of kindled rats. This discrepancy may be explained by differences in the binding procedure; Savage et al. [23] used 100 nM [^{3}H]KA and did not expose the slices after incubation to unlabeled ligand to remove the rapidly dissociating low-affinity binding sites [14] It is therefore possible that these authors evaluated in their study changes in low-affinity ($K_d = 27$–66 nM) rather than in high-affinity ($K_d = 4$–16 nM) KA binding sites. In fact, a recent study by the same group does indicate that this is the case [19]

In a parallel study (see companion paper [20]) we have found a similar increase in the density of KA binding sites in the hippocampus of epileptic children as compared to age-matched controls, in the fascia dentata and the region CA3

The mechanism of this sprouting is presently unknown. However, it is clear that the increased density of KA receptors will promote further excitability of the hippocampal circuitry The epileptogenic properties of KA are due to its powerful excitatory effects [1], to a modification in the synaptic properties of CA3 pyramidal neurons [2] and to a reduction of both $GABA_A$- and $GABA_B$-mediated potentials and several voltage-dependent K^+ conductances (notably I_Q and I_{AHP} [3, 7, 10] The following observations suggest that the aberrant granule–granule cell connection is functional and may participate in the maintenance of epileptic disorders· (1) in slices from KA-treated rats which show an aberrant supragranular band of mossy fibers a single stimulus of mossy fibers induces repetitive discharge in these neurons [25], (2) animals which exhibited chronic seizures after KA treatment had significantly more supragranular sprouting of mossy fibers than animals which did not have seizures [5]

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