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An experimental model of progressive epilepsy: the development of kindling of the hippocampus of the rat

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Kindling epileptogenesis was induced by periodic electrical stimulation of the Schaffer collateral/commissural pathway in the CA1 area of the rat hippocampus. The progressive nature of hippocampal kindling is demonstrated by a detailed description of the behavioral signs and the progressive increase of the after-discharge duration in the course of kindling acquisition. Furthermore, the evolution of the alterations in the paired-pulse local evoked field potentials and the modifications of the $GABA_A$ receptor binding and of the expression of mRNAs encoding for the subunits of the $GABA_A$ and glutamate receptors are considered. Evidence is presented that during kindling opposite changes occur in the CA1 and the fascia dentata in terms of the balance between excitation and inhibition due to contrasting changes in GABA-mediated inhibitory function.

Key Words: kindling — epilepsy — hippocampus — field potentials — inhibition — $GABA_A$ receptor — mRNA expression — in situ hybridization.

The stimulation of certain pathways of the brain with a short train of electrical pulses, repeated at regular intervals, causes progressive changes in the underlying neuronal networks. These changes become manifest as after-discharges (ADs) of progressively longer duration. At a given stage, these ADs propagate to related brain areas and eventually lead to the occurrence of seizures of focal onset that can become generalized. [9], the first researcher to describe this phenomenon named it kindling, a designation that describes in a striking manner the progressive character of the development of this type of epileptogenic focus. We choose to investigate the basic mechanisms responsible for kindling epileptogenesis in the CA1 area of the hippocampus of the rat. With this aim in mind, we stimulated the Schaffer collateral/commisural fibre bundle in the dorsal hippocampus.

In this review, we present evidence for the progressive character of kindling epileptogenesis, in an attempt to construct a flow path of the successive changes that underlie this form of epileptogenesis. Here, we concentrate on an analysis of the processes that lead to the development of the kindled state and thus do not consider the changes that are still present at long-term, i.e. many weeks or months after the last kindling stimulations.

The general methodology used was as follows: male Wistar rats (250-400 g), under anesthesia, were implanted with stainless steel electrodes (80 μm diameter) for stimulation and recording using stereotaxic coordinates, in addition to reference and ground electrodes (screws implanted in the

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skull). Recordings were made from the stratum radiatum of the CA1 area (coordinates: 2.4 mm posterior to the bregma and 1.4 mm lateral to the midline). Stimulating electrodes were placed in the Schaffer collateral fibre layer (coordinates: 2.8 mm posterior to the bregma and 2.4 mm lateral). In some experiments, we used additional sets of electrodes to measure changes in another area within the hippocampus, the fascia dentata (FD); namely stimulation electrodes were placed in the angular bundle (AB) (coordinates: 7.8 mm posterior to bregma, 4.4 mm lateral), and recording electrodes in the hilar region of FD (coordinates: 2.8 mm posterior to the bregma and 2.4 mm lateral). The kindling procedure consisted of applying twice daily trains of 1 s duration (50 Hz, 200-300 μA, 0.1 ms pulse duration) to the Schaffer collaterals. These procedures have been described in detail elsewhere [11, 14].

In this review, we present the experimental data that have been collected concerning the acquisition of the kindled epileptogenic focus, i.e., during the phase when the behavioral signs and the corresponding electrophysiological phenomena elicited by the kindling procedure become increasingly manifest. This developmental phase lasts, in general, 20-25 sessions. We have given especial attention were to the development of the behavioural signs during kindling of the hippocampus since this question is usually treated in a rather superficial way in the literature. The following aspects are considered:

(a) unfolding of behavioral signs and development of after-discharges (ADs)
(b) evolution of paired-pulse evoked potentials
(c) modifications of $GABA_A$ receptor binding and of the expression of mRNAs for the subunits of the $GABA_A$ and the glutamate receptors.

(a) Unfolding and behavioural signs and development of after-discharges (ADs)

Hippocampal kindling seizures evolve in the course of kindling, from an almost imperceptible change in behavior to complete tonic/clonic convulsions. Parallel local EEG changes show a continuous increase of the duration of the after-discharges. Racine [28, 29] has introduced a scale, from zero to 5, to characterise the progressively severe behavioral signs that appear in the course of kindling seizures. In the case of kindling of the Schaffer collaterals in CA1 area we observed that at the start the rats do not show any behavioral sign that may be considered as a manifestation of seizure (stage 0). After a few stimulations, the animals can present a few twitches of a wiskers, an arrest reaction that may persist for 10-20 seconds after the stimulus train and "wet dog shakes" (stage 1; thereafter rhythmic contractions of the eyelids and other muscles of the nostrils appear (stage 2); later movements of the jaw (chewing) and of the neck (head bobbing) (stage 3); still later forelimb clonic contractions can occur with rearing (stage 4); at a final stage, the contractions spread over the whole body, involve the rear limbs such that the animals loose posture (stage 5). It is common to use these scores (stage 0 to 5) to quantify the animals' behavioural seizures. After the generalized seizures, the animals can be disoriented for a period of minutes. When a number of generalized seizures (stage 5), for instance 5 or more, occurred the rat is said to have reached the kindled state. Fully kindled animals that were left unstimulated for long periods of time, even as long as six months, can respond to a renewed stimulation with a generalized convulsion.

The unfolding of the different behavioural stages for a group of animals can be seen in Fig. 1. It is important to note that the session at which the first stage 5 seizure occurs is variable as illustrated in Fig. 2. Fig. 3 presents the evolution of the duration of the corresponding EEG after discharges (ADs). An observation of Fig. 1 and 3 indicates that the occurence of generalized convulsions (stage 5) accompanies the increase in duration of the ADs, but the relationship between both is not directly evident. This can be made clearer when the means of the duration of the ADs corresponding to the different seizure stages are compared. For this comparison we computed the distribution of the ADs for different seizure stages; examples for stages 2 and 5 are shown in Fig. 4a and 4b. The means and s.e.m. of the ADs for the different stages are presented in Table I. It can be concluded that there is a significant increase of the AD duration with seizure stage, even with the same kindling period, that in this case was chosen between session 11 and 20, where the first stage 5 seizures start to occur. For this statistical comparison stages 3 and 4 were combined because these occur in relatively small numbers, and the seizure type was the independent variable considering that the different seizure stages were presented by all 38 animals studied.

The number of stimuli needed to reach the kindled state depends, of course, on the parameters of stimulation and on the brain site being stimulated. The amygdala is more sensitive than the hippocampus. While the amygdala needs only about 10 stimulation sessions to reach the kindled state, kindling of the hippocampus elicitis the first stage 5 seizure on average, for the rats sudied here, in 19±17 session. Another important parameter is the interval between the stimulation sessions. Recently, Lothman and Williamson [22] compared the effects of "rapid kindling", a protocol consisting of blocks of 12 supramaximal

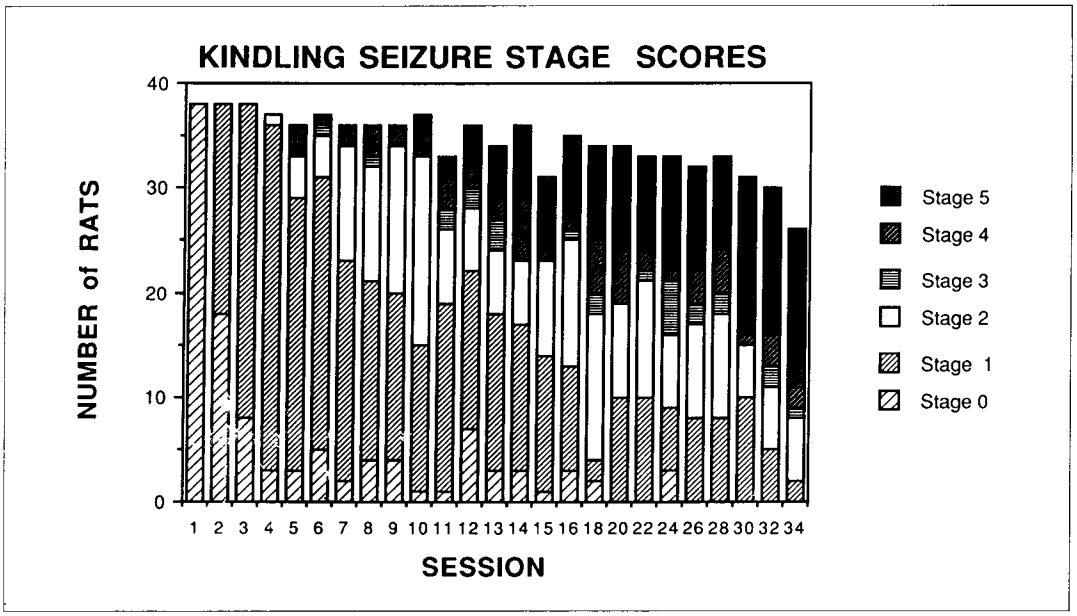


Fig. 1. *A. Stack histogram representing the distribution of the behavioral scores obtained in the course of kindling for a group containing initially 38 rats. The number of rats tested at every session is not always the same due to technical reasons. Note that the number of rats presenting no behavioral signs after the tetanic stimulation (stage 0, for the definition of stages see text) decreases sharply at the beginning of kindling and that manifestation typical of stage 1 dominate in the first week; stage 2 develops after 6 or 7 sessions and it is scored regularly during the whole observation period; stages 3 and 4 are scored in a relatively small number of cases. Although at the end of the observation period a majority of rats present seizures at stages 4 and 5, there is still some cases where stages 1 and 2 are scored.*

trains per day, with interstimulus intervals of 30 min., in consecutive days until 48 trains where given, with different protocols of what they called "slow kindling", among which also a protocol similar to that we describe here and that we may call "classic kindling". The latter protocols have the common characteristic that the stimulus trains are given at relatively long intervals and are spread over periods of one week or more. These protocols may result in persistent fully kindled states, in contrast to the "rapid kindling" where this is not observed. This finding implies that the mechanisms underlying the development of kindling need time to develop, what suggests that slowly developing molecular processes are likely involved in kindling.

(b) Evolution of paired-pulse evoked potentials

Changes in excitability of neuronal networks in the course of the development of kindling can be assessed easily by recording local field potentials (EPs) at regular intervals. These EPs are the extracellular reflection of post-synaptic potentials and other events, such as compound action potentials (population spikes). To evalutate excitability, it is convenient to use a paired-pulse paradigm. In order to quantify the interaction between the responses to a pair of stimuli given at short intervals, the peak amplitude of the response to the second pulse of the pair is divided by the response to the first. We call this parameter the paired-pulse index (PPI). The value of the PPI depends on the intensity of the stimuli and on the interval between them. Typically, at low stimulation intensities and at inter-pulse intervals around 20 ms, the PPI presents a value larger than one, indicating the existence of paired-pulse facilitation (PPF). However, at larger intensities, the PPI becomes smaller than one, and thus PPD (paired-pulse depression) can be said to be present. This occurs when the second stimulus is given during an IPSP.

Kindlling of the Schaffer collaterals/ commissural fibers is accompained by a reduction of PPD of the CA1 responses [14], as illustrated in Fig. 5a. Similar results were obtained by Zhao and Leung [47, 48]. Taking into consideration that this result was at odds with indications that an enhanced PPD due to kindling could be found in other areas of the hippocampus, namely the fascia dentata (FD) [7, 24, 26, 31, 41, 42], we investigated, in the same animals, whether regional differences in PPI,

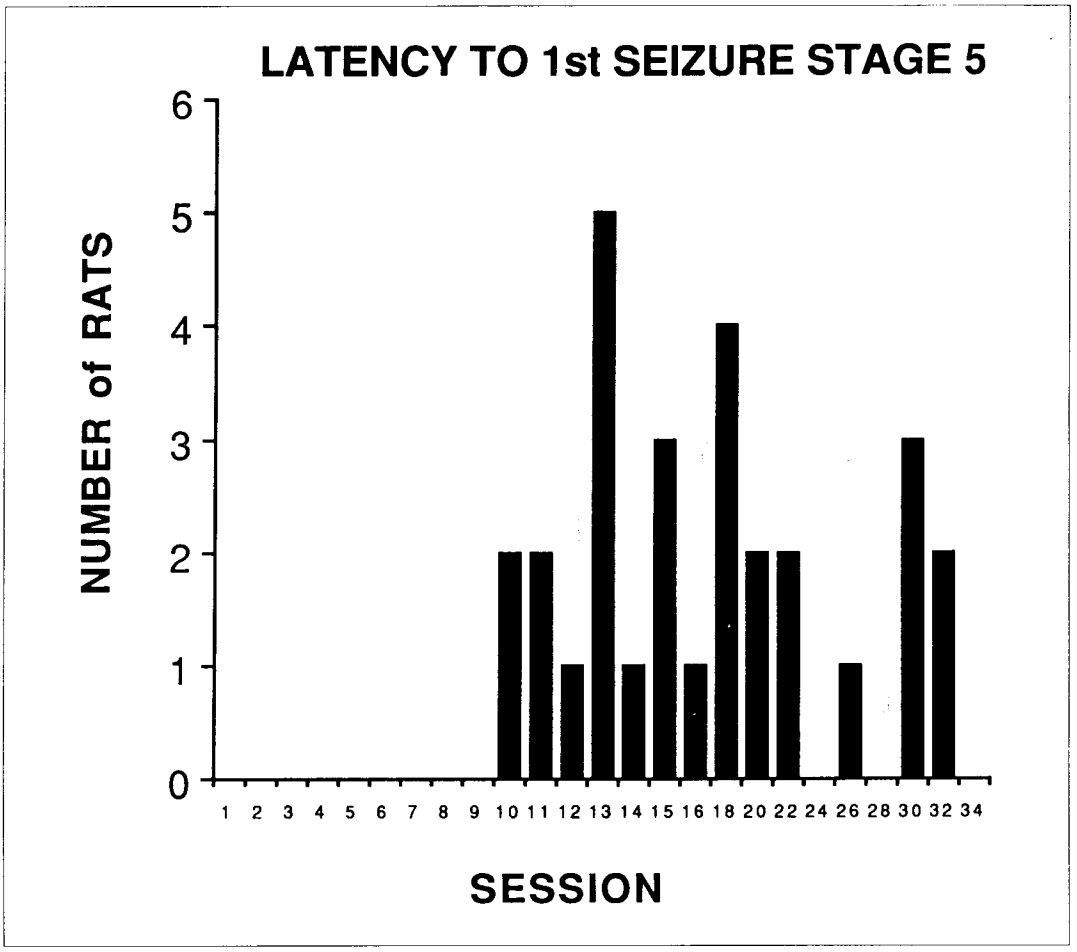


Fig. 2. *Distribution of the 1st stage 5 seizures along the sessions, for each rat. Note that the first two stage 5 seizures were scored at session 10, but that some rats had their first stage 5 seizure only after more than 30 sessions.*

could occur within the hippocampus during kindling of the Schaffer collaterals/commissural pathway (SCH). EPs were elicited in CA1 and fascia dentata areas by stimulating, respectively, the SCH and of the AB fibers to analyse the two systems: SCH->CA1-SR, and AB->FD-HL. Fig. 5b shows that at the intensity given, the AB>FD-HL responses present PPD, and the depression is further accentuated in the course of the development of kindling [11]. This experiment shows that in the same animal, changes in opposite directions to the stimulation of the same pathway can occur in the course of kindling development. It implies that the local neuronal circuits and elements of the CA1 area and the FD react differently to kindling stimulations. In an attempt to understand the basic processes underlying this phenomenon, we carried out a number of studies in both hippocampal areas in relation to the development of kindling, namely studies of receptor binding, functional studies of the GABA receptor in synaptoneurosomes prepared from both areas and in dissociated cells, and *in situ* hybridizations of glutamate and GABA receptor subunits.

(c) Modifications of $GABA_A$ receptor binding and of the expression of mRNAs for GABA and glutamate receptors

A quantitative autoradiographic study of the binding of the GABA agonist [^{3}H]muscimol was carried out in rats that were fully kindled and corresponding controls. The former had experienced an average of 4 class 5 seizures and a mean of 25 after-discharges. The rats were sacrificed at least 24h after the last seizure and autoradiograms of brain sections were made using the ap-

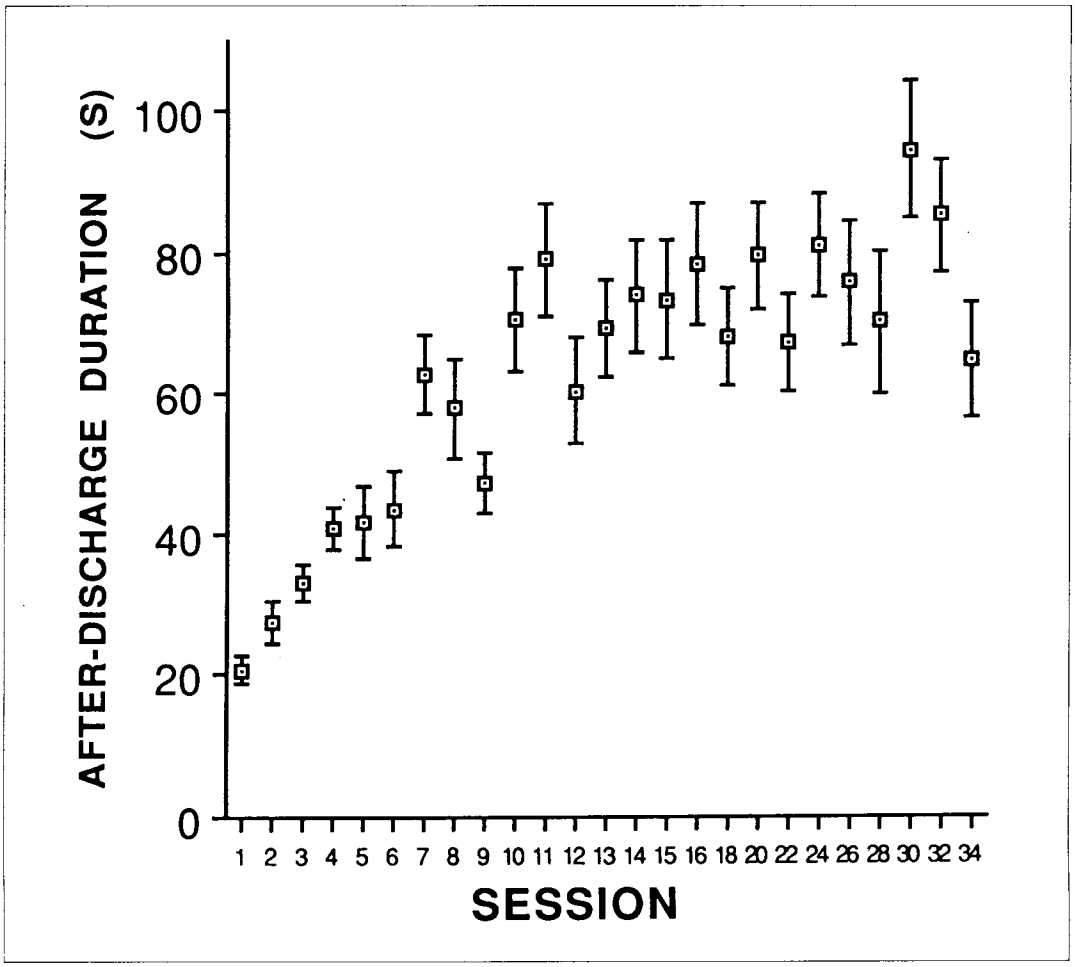


Fig. 3. *Evolution of the duration of after-discharge (ADs) in the course of kindling for the same group of rats of fig. 1; means and s.e.m. are indicated. The parameters of the kindling tetanus were chosen such that at the first session an AD of about 20 s duration was evoked.*

propriate ligands. The detailed methods are described in Titulaer et al. [38]. The main results are presented in Fig. 6. Several concentrations of [^{3}H]muscimol were used in the range of the high/intermediate-affinity (5-40 nM) and low-affinity (60-100 nM) binding sites. In the fully kindled rats the binding was significantly increased by 20-30% in the FD, while it was decreased in the CA areas by 10-25%. It is noteworthy that the changes in [^{3}H]muscimol binding, the opposite was the case for the FD: i.e. an *increase* in PPD corresponded to an *increase* in [^{3}H]muscimol binding. Approximately similar changes, both for the CA area and the FD, were found for the binding of the benzodiazepine receptor site, using [^{3}H]flunitrazepam and foor the "cage convulsant" t-butylbicyclophosphorothionate ([^{35}S]TBPS) that binds to the Cl-channel site.

Both the electrophysiological observation using PPI as parameter, and the autoradiographical $GABA_A$-receptor binding studies are indicative that the development of kindling is accompained in the *CA1 area* by a *decrease* in $GABA_A$ ergic inhibition. With regard to the changes in the *FD* there is also agreement between the increases in PPD, indicating an *increase* in $GABA_A$ ergic inhibition and the increase in binding of the $GABA_A$ receptor sites found in the autoradiographical studies.

A comprehensive investigation using in situ hybridization to detect mRNAs levels for the $GABA_A$ receptor in the FD at the fully kindled stage. Namely the α_1, α_2, α_4, β_1, β_2, β_3 and the γ_2 subunits showed all significant increases (>20%) in mRNA expression, but in CA area a quite differ-

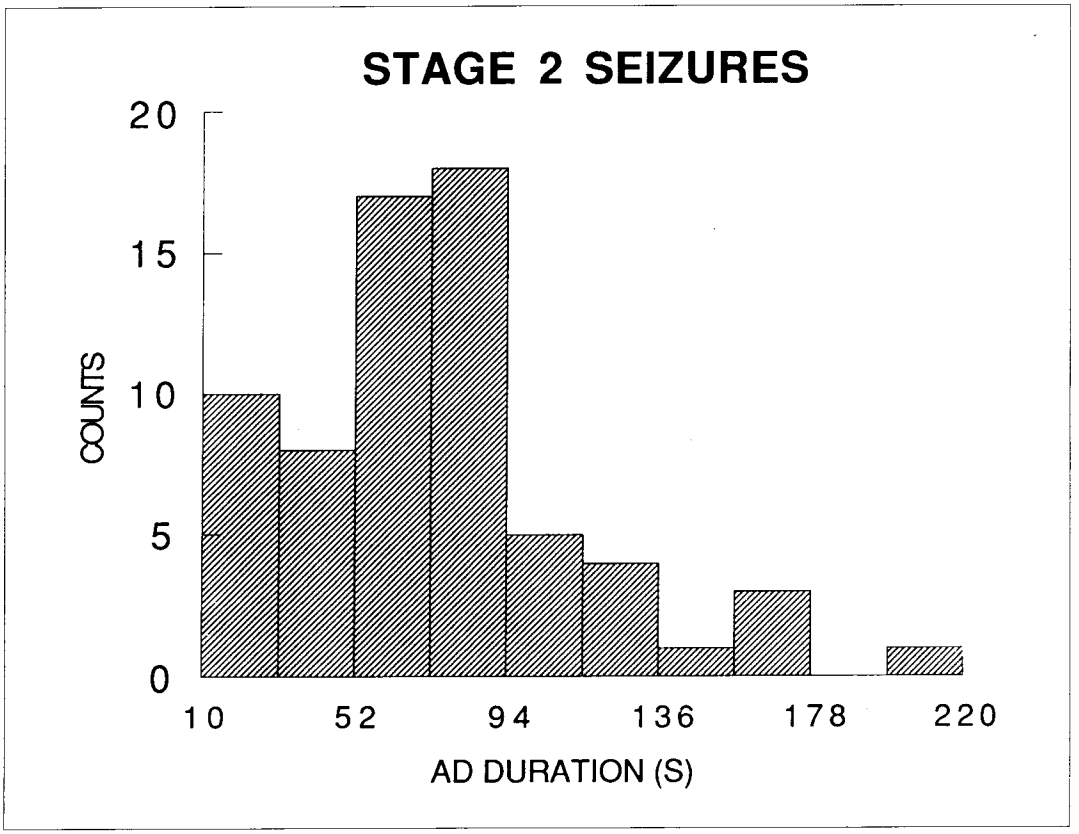


Fig. 4a.

ent picture was obtained: there were just some small increases of α_2 and γ_2 accompanied by a small decrease of β_2 [10].
In addition to these changes in $GABA_A$ receptors, we found that kindling also induced changes in the expression of the AMPA type of glutamate receptors, namely of the mRNAs encoding for the Flip and Flop variants of the receptor subunits A, B, and C [15]. In the FD there was a conspicuous increase of the Flip variant without change of the Flop variant. In the CA1 similar changes were not found, only a small decrease of the Flip variant was noted for some time samples in the course of kindling. We discuss below the possibile physiological implications of these findings.

(d) Functional studies of the GABA receptor in vivo, in synaptoneurosomes and in CA1 pyramidal neurons using the patch-clamp technique

A first functional study of GABAergic neurotransmission was carried out in vivo using extracellular recordings of single unit activity recorded from the pyramidal layer of CA1 area [12]. These neurons were excited by the iontophoretic application of glutamate; subsequently GABA was ejected and the ensuing decrease in firing rate was determined. We found a decrease in the response to GABA of these neurons. This findings is in line with the changes that were encountered in GABA binding sites using autoradiography. However, similar studies were not performed in the FD, so that we are not able to conclude whether the two hippocampal areas behave in opposite directions.
In order to investigate the sensitivity of the GABA receptor used isolated synaptoneurosomes, which contain the presynaptic terminal and the attached postsynaptic closed membrane structures. They were isolated separately from CA and FD areas, and we determined the $GABA_A$ mediated ^{36}Cl-fluxes using muscimol, both for controls and fully kindled animals. In the latter animals, the muscimol-stimulated ^{36}Cl-uptake was significantly reduced by 21% in the CA area whereas a significant increase of 29% was found in the FD. Thus these findings are in close agreement with the autoradiographic binding data and

the results of the paired-pulse electrophysiological observations.
It was possible to measure $GABA_A$ mediated Cl-currents, in acutely isolated CA1 pyramidal neurons from fully kindled rats and from the corresponding controls [43]. Using the whole-cell voltage-clamp technique, no change was found in the maximal Cl-current amplitude; the time-constant of desensitisation of the $GABA_A$ receptor was the same in kindled and controls. In addition, it was found that Ca^{2+} influx, due to activation of voltage-dependent calcium currents, decreased appreciably the muscimol-evoked Cl-current. It is interesting to note that kindling induces an enhancement of voltage-dependent calcium currents when investigated in dissociated pyramidal CA1 cells [44] as well as in slices using the in situ patch-clamp technique [8]. Indeed, it is known that the $GABA_A$-receptor is modulated by calcium-dependent phosphorylation processes [5, 20, 36]. Therefore, it is likely that the increased voltage-dependent Ca^{2+} currents in the CA1 neurons may cause a down-modulation of GABA mediated Cl-, and thus in the indirect way a decrease in inhibitory transmission.

TABLE I.

Mean ± s.e.m. of the duration (s) of the after-discharges for different seizures stages for sessions 11-20			
Stage 1	Stage 2	Stage 3 & 4	Stage 5
64.2±4.2 (2=60)	75.4±4.6 (n=67)	99.5±6.9 (n=35)	118.2±5.2 (n=50)
p<0.004p<0.03			

Discussion and conclusions

Kindling of the hippocampus is a model of epileptogenesis that permits the study of how the development of an epileptogenic focus gradually takes place in the course of time. The gradual enhancement of the duration of after-discharges develops in parallel with the unfolding of succes-

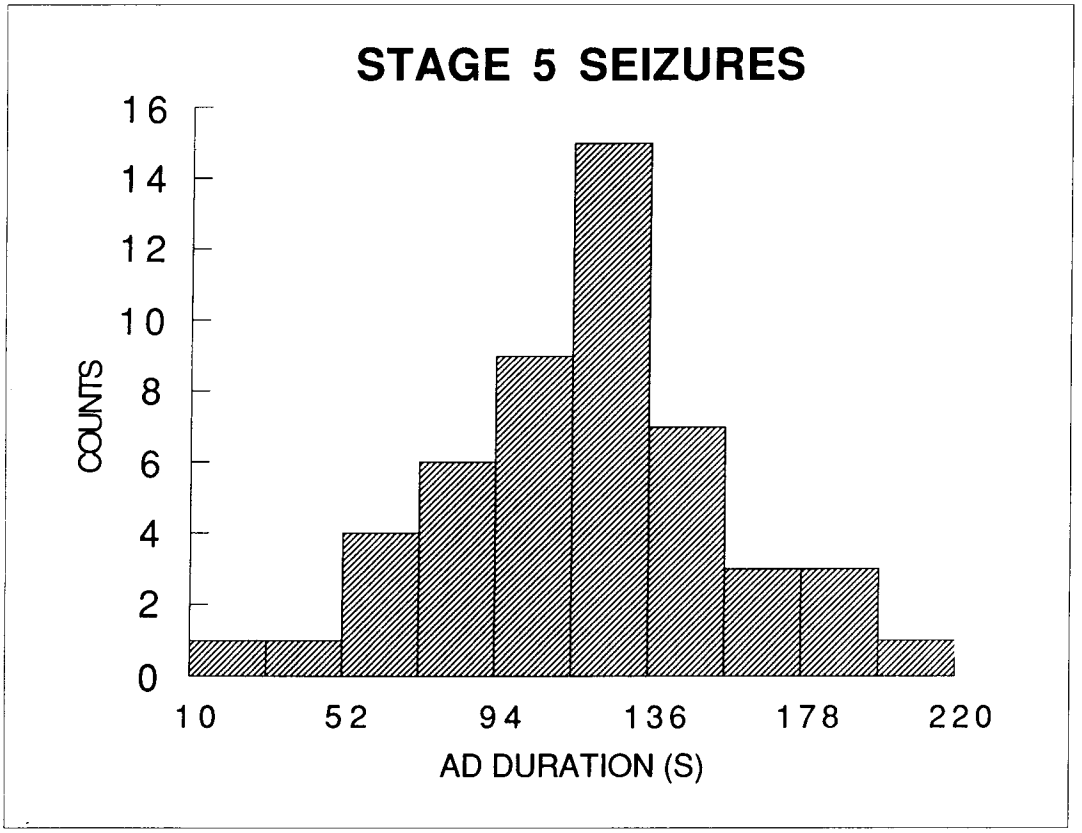


Fig. 4b. *Distributions of the durations (in sec) of the ADs corresponding to stage 2 and stage 5 that were scored in the session 11 to 20. For the statistical comparison see Table 1.*

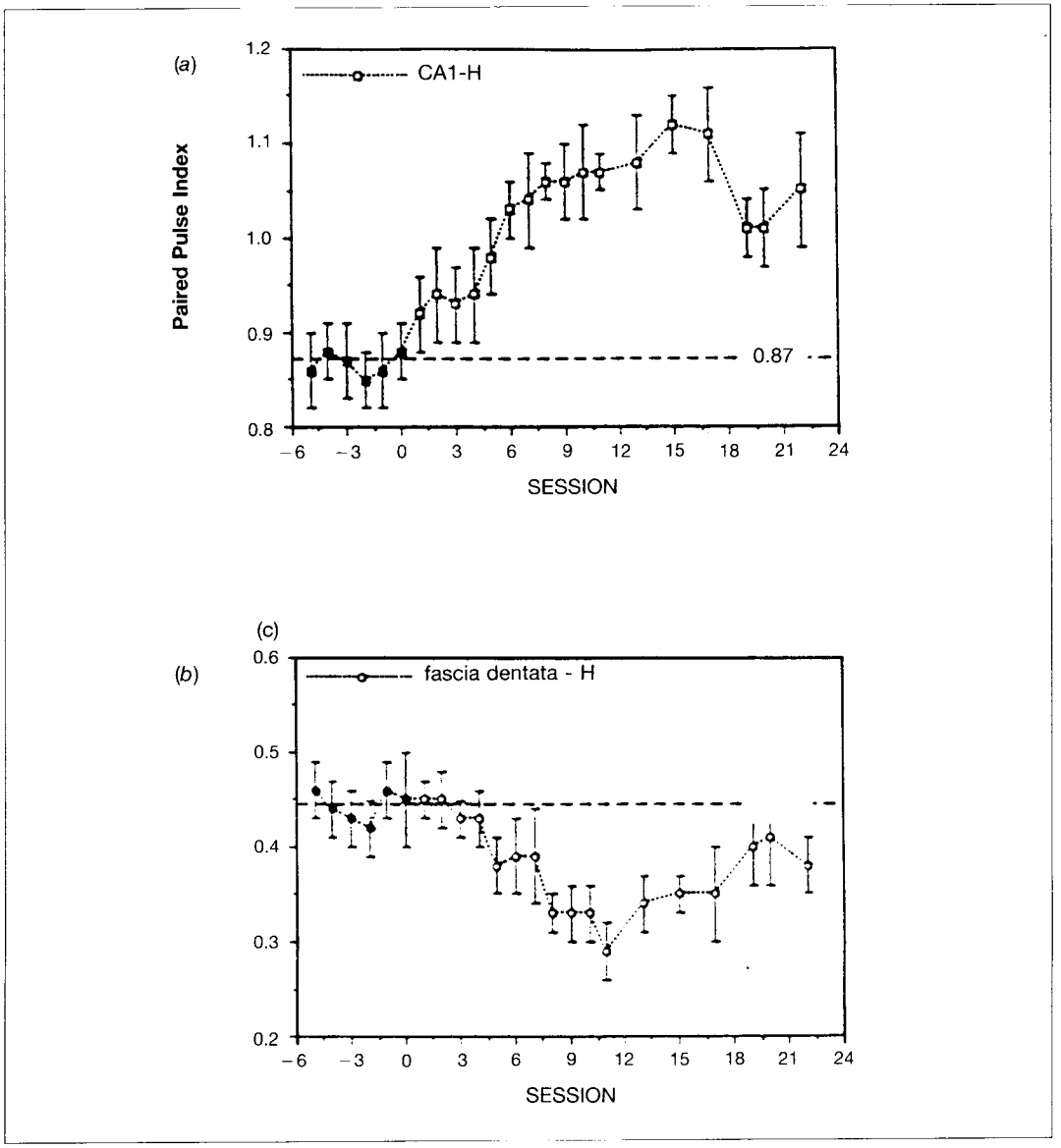
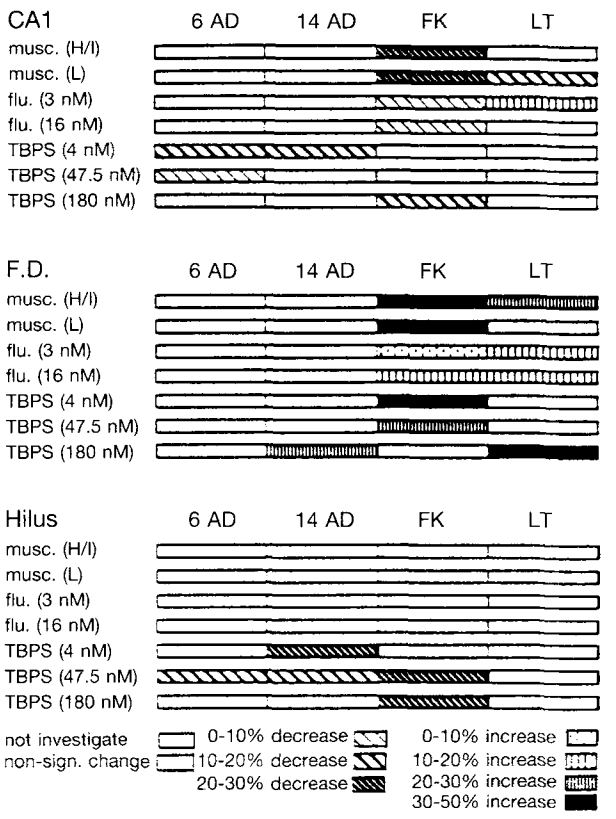


Fig. 5a e b. *Development of paired-pulse index (PPI) in response to strong stimuli applied to (a) the Schaffer collaterals in CA1 (SCH->SR) and (b) to the angular bundle in FD (AB->HL) for the same group of rats of Fig. 1 (Adapted from Kamphuis et al. 1992).*

sively more severe behavioural seizures. This mean that these two phenomena and related. Since the hippocampus is not an output motor structure but projects to a number of target areas related to the motor systems of the forebrain and to other cortical areas [21], it is likely that a relatively long hippocampal after-discharge is necessary for the activation of these structures in order for behavioral seizures to occur.

The evolution of the after-discharges in the course of kindling is typically accompanied by gradual changes in electrophysiological parameters, which are characteristic of the excitability of the local neuronal networks. The main feature in this respect is the gradual *decrease* of the paired-pulse depression (PPD) in CA1 area. Surprising is the fact that along with this change in CA1, there is an *opposite* change in FD. In general, we may as-



Musc (H/I) = high/intermediate-affinity (5-40 nM) [^{3}H]muscimol binding;
Musc. (L) = low-affinity (60-100 nM) [^{3}H]muscimol binding;
Flu. (3) = 3 nM (K_D value) [^{3}H]flunitrazepam binding;
Flu. (16) = 16 nM (B_{max} value) [^{3}H]flunitrazepam binding;
TBPS (4) = 4 nM [^{35}S]TBPS binding;
TBPS (47.5) = 47.5 (K_D value) [^{35}S]TBPS binding;
TBPS (180) = 180 nM (B_{max} value) [^{35}S]TBPS binding.

Fig. 6. *Summary of the relative changes (%) in $GABA_A$ receptor ligand binding during kindling at different stages: after 6 ADs, 14 ADs, after at least 5 stage 5 seizures (Fully Kindled or FK) and 28 days after the last kindling stimulus (Long-term or LT) (Adapted from Titulaer 1994). Note that the significant changes found in CA1 and in the hilar region were decreases (except for an increase in flunitrazepam binding (3 nM) at long-term), whereas in FD all of these changes were increases.*

sume that PPD is a measure of the balance between excitatory (E) and inhibitory (I) processes, i.e. of the *E/I index* that can be considered a main control parameter of the local network. The changes in PPD indicate that a relative increase of the E/I index occurs in CA1, while in the FD there is a decrease in this index. Thus within the hippocampus, the process of epileptogenesis is associated with different, even opposite, changes in PPD. This findings merits further discussion. In this respect, a number of point should be made.

First, a large amount of experimental data and theoretical studies [40] leads to the hypothesis that epileptogenesis is, in general, induced by an increase in E/I index. Therefore, the change in CA1 found in our kindling model in compatible with such an hypothesis. However, this does not apply to the FD. The functional consequences of these opposite changes in CA1 and FD for the excitability of the limbic circuits involved are quite different. This can be put in evidence considering that from the functional point of view, these two

hippocampal areas occupy apart positions within the hippocampal circuits; while the FD occupies the position of input interface, the CA1 areas is an output structure. Therefore, an increase in excitability in the latter will have more direct consequences to the activity of target structures, and thus may lead to the propagation of seizure activity over several brain areas. The change in the FD, that appears to correspond to an enhancement of inhibition within the local network, could lead to a stronger inhibition of the inputs converging into the FD from the neocortex through the entorhinal cortex [46]. Consequently, the changes occurring within the FD during kindling will reinforce the working of the latter as an inhibitory shield. A point we should discuss here is whether the apparent increase in inhibition in the FD may be related to the phenomenon of *sprouting*, conspicuous in the latter, but not in the CA1. Sprouting involves not only the mossy fiber system that form new excitatory synapses in the inner thirds of the molecular layer of the FD and in the infrapyramidal layer of area CA3 [4, 30] but also local inhibitory synapses [6]. The sprouting process in the FD may be considered a reactive mechanism due to a neuronal loss in the hilus of the FD, mainly of somastatine/NPY containing subpopulation of interneurons, while terminals of NPY-immmunoreactive interneurons, that are presumed inhibitory, may also sprout into the molecular layer [1, 34]. However, the importance of sprouting for the process of epileptogenesis is not yet settled, considering that Sperber et al. [33] did not find signs of sprouting in rat pups after the induction of epileptic seizures by kindling, kainic acid or fluorethyl. Thus sprouting, at least in the very young rat, is not a prerequisite for the development of kindling epileptogenesis.

Second, the finding that the PPD measured in the CA1 and in the FD clearly changes in opposite directions, leads directly to the question whether this difference depends on cellular processes that also change in opposite directions within the same hippocampus. Although a complete set of cellular and molecular phenomena studied in both structures, using the same kindling protocol, is not yet available, a number of findings can be considered in this respect, namely (a) $GABA_A$ receptor binding, (b) the GABA mediated Cl-fluxes in synaptoneurosomes and the corresponding currents recorded in isolated neurons, (c) the calcium currents, and (d) the expression of mRNAs for glutamate and $GABA_A$ receptors:

(a) The density of all three ligands of the $GABA_A$ receptor studied, i.e. of muscimol binding sites of the high/intermediate affinity class, of benzodiazepine binding sites (flunitrazepam binding) and the TBPS (Cl-channel) sites changes indeed in opposites directions, decreasing in CA1 and increasing in FD [37, 38, 39].

(b) The muscimol-activated Cl-fluxes in synaptoneurosomes changes also in opposite directions, decreasing in CA1 and increasing in FD [37]. The direct measurements of ionic current present a more complex picture. Vreugdenhil [43] found no change in muscimol activated Cl-currents in acutely dissociated CA1 pyramidal neurons. However, Otis et al. [27] reported an increase in GABA mediate inhibitory currents (IPSCs) of granule cells in FD after local kindling. Thus in the FD, there is agreement between the results of synaptomeurosomes and the whole-cell patch studies, but this does not appear to be the case for the CA1. We should point out that a direct comparison between the results obtained using synaptoneurosomes and dissociated neurons is not simple, because the former are obtained from the whole CA tissue and include all types of synaptic structures within this hippocampal area, while the latter represent dissociated pyramidal cells that are limited to the soma and proximal dendrites. Assuming that this methodological difference can explain the different results obtained with the synaptoneurosome and the patch-clamp studies in CA1, we may interpret these findings as indicating that the synaptic structures contributing to the results of the synaptoneurosome studies correspond mainly to those locate at the dendrites, and that these are not included in the measurements carried out in dissociated neurons.

Taking the several aspects discussed above into consideration, it may be concluded that with respect to $GABA_A$-mediated currents, also some changes take place in opposite directions in CA1 and FD.

(c) There are, as yet, no measurements of Ca^{2+} currents made both in CA1 and FD cells in the same preparation and kindling model. Vreugdenhil and Wadman [44] found a significant increase in Ca^{2+} currents (substained and slow-inactivating components) in dissociated CA1 pyramidal neurons at the fully kindling stage. In contrast, Köhr and Mody [17] reported that kindling did not affect Ca^{2+} currents measured in isolated granule cell of the FD, but increased their calcium-dependent inactivation probably due to a loss of calcium binding proteins. We may add, in relation to these observations, that in the course of kindling the interneuron population is not affected in an equal manner. In our model, we found that the GABA immunoreactivity decreased, but exclusively for those cell where GABA did not co-localize with the calcium-binding protein parvalbumin [13], indicating that the GABA containing cells that were unaffected by kindling were those protected from an increase in intracellular Ca^{2+} by

parvalbumin. Recently, it was shown that the GABAergic interneurons that make synaptic contact with the perisomatic surface of CA pyramidal neurons contain parvalbumin, whereas this calcium-binding protein is absent in those interneurons innervating apical dendrites, which may contain other proteins [16, 25]. Therefore also as regards Ca^{2+} currents, and possibly also in Ca^{2+} buffering, there appear to exist differences between CA and FD cells.

(d) Semi-quantitative *in situ* hybridization techniques to determine levels of mRNAs encoding for specific subunits of glutamate and $GABA_A$ receptors in the kindled hippocampus, also revealed clear differences between CA area and FD. The expression level of the mRNAs encoding for the Flip version of the AMPA-receptor subunits A-, B-, and C- was appreciably increased already after 6 after-discharges, i.e. before overt seizures were detected, and also in fully kindled rats, in the granule cells of FD; whereas in CA1 only some very small decreases were found at initial stages. Furthermore, a number of $GABA_A$-receptor subunit were appreciably increased in FD, but the changes in CA area were slight; only in the case of one subunit β_2, a change in opposite direction, i.e. an increase in FD and a decrease in CA3 area, was encountered but this effect was discrete [10].

What are the possible implications of these changes in relation of kindling?

First, we consider the changes in glutamate receptors. In the granule cells of the FD, we observed a shift in the ratio of Flip- to Flop-carrying subunits which may lead to an enhanced response of the corresponding receptors to L-glutamate and a less desensitizing excitatory response, according to the studies of Sommer et al., [32] and Burnashev, [3]. This is compatible with the observation that a long-lasting potentiation of the excitatory synaptic response takes place in the FD in the course of kindling, while this is not the case in CA1 [24, 35].

Second, the changes in $GABA_A$ receptor mRNAs indicate an up-regulation of the number of these receptors, since the subunits that presented the largest changes correspond to those that are most abundant under normal conditions. Of course these changes in mRNA expression correlate closely with the enhanced [^{3}H]muscimol, [^{3}H]flunitrazepam and [^{35}S]TBPS binding, the increased $GABA_A$-mediated Cl^- uptake by synaptosomes prepared from the FD and the gradual strengthening of the PPD in this area. The changes of mRNAs levels in CA areas, although much slighter than those found in FD, are apparently not in line with the changes in receptor binding and in the Cl^- flux measurements in synaptoneurosomes. This was unexpected. At present, we can only speculate about the processes underlying this observation. The finding, as such, may be interpreted as the result of an aberrant translation, or post-translation processing, of the $GABA_A$-receptor subunits. A possible mechanism is that these changes may reflect cellular regulatory processes. Namely, the fact that in the course of kindling the $GABA_A$-receptor function, in the CA area, is down-regulated due to the increased Ca^{2+} currents and subsequent changes in phosphorylation of the $GABA_A$-receptor, may lead to a compensatory enhancement of mRNA expression, in order to maintain the balance between excitation and inhibition (E/I index) as close as possible to the normal range.

As *conclusion*, we may make an attempt to combine the anatomical data with the physiological findings indicated above, in order to speculate about the significance of the changes encountered for the phenomenon of kindling epileptogenesis. We may formulate the *hypothesis* that the decrease in GABAergic inhibition, revealed by the synaptoneurosome investigation, should be attributed to changes at the inhibitory synapses locally *distally* from the cell bodies, namely those situated in the stratum radiatum the laconosum-moleculare of CA1 and may be also in stratum oriens. In addition, the fact that the dissociated pyramidal cells present an increase in voltage-dependent Ca^{2+} currents (sustained and slow-inactivating components, [44, 45], along with the finding that Ca^{2+} influx depresses $GABA_A$ mediated Cl^- currents, indicates also that GABAergic inhibition may be depressed in these cells depending on their activity state. In this context it should be noted that the CA1 interneurons that are affected by kindling are those where GABA does not co-localize with parvalbumin and are situated in the strata radiatum and laconosum-moleculare of CA1 (Buhl et al 1994). These interneurons have been considered to be mainly responsible for feedforward inhibition [18, 19], but some appear also to receive collaterals of pyramidal cells and thus may subserve feedback inhibition such as the bistratified interneurons [2] that make synapses at the same level as the SCH collaterals. Therefore, it appears that one of the inhibitory control mechanisms of the excitability of the neuronal populations of the CA1 may be critically affected in this area in the course of kindling of the SCH/commissural pathways.

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Sommario

Mediante stimolazione elettrica periodica della via commissurale/collaterale di Schaffer nell'area CA1 dell'ippocampo di ratto è stata indotta una epilessia legata al meccanismo del "kindling". La natura progressiva del fenomeno è dimostrata da una dettagliata descrizione di segni comportamentali e del progressivo incremento della durata della scarica durante il "kindling". In aggiunta sono state considerate: l'evoluzione delle alterazioni dei potenziali di campo evocati da coppie di pulsi, le modificazioni del legame recettoriale per i $GABA_A$, l'espressione dell'mRNAs che codifica per le subunità recettoriali del $GABA_A$ e Glutamato. Vengono qui presentate le evidenze secondo cui durante il kindling avvengono in CA1 e nella fascia dentata modificazioni opposte in termini di bilancio tra eccitazione ed inibizione dovuti a cambiamenti contrastanti della funzione inibitoria GABA-mediata.

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