Miniature Inhibitory Postsynaptic Currents in CA1 Pyramidal Neurons After Kindling Epileptogenesis

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Wierenga, Corette J. and Wytse J. Wadman. Miniature inhibitory postsynaptic currents in CA1 pyramidal neurons after kindling epileptogenesis. J. Neurophysiol. 82: 1352–1362, 1999. Miniature inhibitory postsynaptic currents (mIPSCs) were measured in CA1 pyramidal neurons from long-term kindled rats (>6 weeks after they reached the stage of generalized seizures) and compared with controls. A large reduction in the number of mIPSCs was observed in a special group of large mIPSCs (amplitude >75 pA). The frequency of mIPSCs in this group was reduced from 0.042 Hz in controls to 0.027 Hz in the kindled animals. The reduction in this group resulted in a highly significant difference in the amplitude distributions. A distinction was made between fast mIPSCs (rise time <2.8 ms) and slow mIPSCs. Fast mIPSCs, which could originate from synapses onto the soma and proximal dendrites, had significantly larger amplitudes than slow mIPSCs, which could originate from more distal synapses (35.4 ± 1.1 vs. 26.2 ± 0.4 pA in the kindled group; means ± SE). The difference in the value of the mean of all amplitudes and frequency of fast and slow mIPSCs did not reach significance when the kindled group was compared with controls. The mIPSC kinetics were not different after kindling, from which we conclude that the receptor properties had not changed. Nonstationary noise analysis of the largest mIPSCs suggested that the single-channel conductance and the number of postsynaptic receptors was similar in the kindled and control groups. Our results suggest a 40–50% reduction in a small fraction of (peri-)somatic synapses with large or complex postsynaptic structure after kindling. This functionally relevant reduction may be related to previously observed loss of a specific class of interneurons. Our findings are consistent with a reduction in inhibitory drive in the CA1 area. Such a reduction could underlie the enhanced seizure susceptibility after kindling epileptogenesis.

INTRODUCTION

Alteration of the strength of synapses between cells is an important mechanism for plasticity in the brain. This plasticity provides the nervous system with the possibility to store (new) information, but if not adequately controlled, it also can lead to changes in the dynamics of neuronal networks that underlie pathological states such as epilepsy (Goddard et al. 1969; Racine 1972). In the kindling model of epilepsy, daily tetanic stimulations of specific afferents result in an epileptic focus and the generation of afterdischarges in the neuronal assemblies in the projection area. Behavioral convulsions appear and gradually increase in severity and duration. The changes in the network seem to be persistent: months after the last stimulation was given, a short tetanus will still induce a generalized convolution. This altered state of the network is usually called the kindled state. A characterization of the kindled state is important for gaining insight into the pathology of epilepsy.

During epileptogenesis the balance between excitation and inhibition, which is essential to maintain stability in a neuronal network, shifts in favor of excitation. Several of the factors that determine glutamatergic and GABAergic synaptic transmission in the CA1 area are changed after kindling epileptogenesis. Previous work demonstrated that the binding of the GABA agonist muscimol is decreased (Titulaer et al. 1994) and that a specific change in GABA_A receptor subunit mRNA occurs (Kamphuis et al. 1995). After kindling epileptogenesis the effective inhibition in the hippocampal CA1 network as judged from paired pulse inhibition is reduced (Kamphuis et al. 1988; Zhao and Leung 1993) and 50% of the GABAergic interneurons that do not contain parvalbumin are lost (Kamphuis et al. 1989). The precise consequences of this reorganization are not known, but the functional effect is a reduced level of inhibition.

The strength of functional inhibition is determined by many factors. In a central synapse, the receptors opposite each bouton are thought to be largely saturated by the release of a single vesicle of transmitter, so that the number of available receptors rather than the amount of transmitter released determines the quantal amplitude (Edwards et al. 1990; Jonas et al. 1993; Nusser et al. 1997). The number of active zones per synapse is also important for determining synaptic strength. The time course of the inhibitory postsynaptic current (IPSC) is largely determined by the receptor kinetics and the release process (Borst et al. 1994; De Koninck and Mody 1994; Glavinovic and Rabie 1998).

The change in network excitability induced by the kindling protocol is persistent and leads to the kindled state. We used long-term kindled rats (the last stimulation that resulted in a generalized seizure was given >6 weeks before the slice experiments) to prevent interference with phenomena that are a direct result from the seizures. We investigated in this study the long-term change in inhibition at the synapse level by measuring miniature IPSCs (mIPSCs) in CA1 pyramidal neurons in hippocampal slices. The mIPSCs are small currents that occur due to the spontaneous release (without a presynaptic action potential) of one (or more) GABA containing transmitter vesicle(s) by presynaptic terminals.

The mIPSCs reflect properties of the underlying GABAergic synapses (Faber et al. 1998). Edwards presented a model of plasticity of central synaptic transmission in which an altered synapse morphology leads to a change in miniature amplitude distribution (Edwards 1995). Changes in the number of postsynaptic receptors after kindling should show up in mIPSC

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amplitude, whereas changes in receptor kinetics (for example due to a change in subunit composition) will be reflected in mIPSC kinetics. A change in the number of spontaneous active synapses should be reflected in the mIPSC frequency.

We used the in situ patch-clamp technique to record mIPSCs in CA1 pyramidal neurons in slices from kindled and control rats and analyzed their amplitudes, kinetics and frequency to study possible long-term changes in the inhibitory synapses after kindling epileptogenesis.

**METHODS**

**Kindling**

Under electrophysiological control and pentobarbital anesthesia (65 mg/kg) electrodes were implanted in the dorsal hippocampus of adult male Wistar rats (200–300 g), as described previously in detail (Kamphuis et al. 1988). In total 19 rats were used for this study of which 11 were implanted. Of these animals, eight received twice daily a tetanic stimulation (200–300 μA at 50 Hz for 2 s) onto the Schaffer collaterals to induce epileptogenesis. Evoked potentials were monitored and electroencephalographic (EEG) recordings were made to follow the gradual increase in length and severity of the after-discharges. Behavioral seizures of class V (Racine 1972) were obtained after 27 ± 2 (SE) kindling tetani. The animals were decapitated without anesthesia 6–8 wk after they had reached the fully kindled state (5–6 class V seizures). The control group consisted of eight age-matched and three implanted rats, which had not received tetanic stimulations. Significant differences between the implanted and non-implanted controls were not observed, therefore they will be pooled and referred to as controls. All animals (control and kindled) were handled similarly during the time of the experiments.

**Slice preparation**

After decapitation the brain was removed rapidly and the hippocampus was dissected. With a tissue chopper 300-μm-thick transverse slices were cut, which were incubated at 32°C in artificial cerebrospinal fluid (ACSF) for 1 h. The ACSF contained (in mM): NaCl, 125 KCl, 2.4 MgCl₂, 2 CaCl₂, 1.1 NaH₂PO₄, 26 NaHCO₃, cerebrospinal fluid (ACSF) for 1 h. The ACSF contained (in mM): NaCl, 125 KCl, 2.4 MgCl₂, 2 CaCl₂, 1.1 NaH₂PO₄, 26 NaHCO₃, and 25 d-glucose and was gassed continuously with 95% O₂-5% CO₂ at a temperature at a membrane potential of -70 mV. Series resistance and whole cell capacitance were monitored during the experiments. Series resistance compensation was not used because it introduced unnecessary noise to the signal and currents were too small to produce significant voltage errors over the pipette resistance (4–6 MΩ). The pipettes were filled with intracellular solution containing (in mM): 140 CsCl, 10 EGTA, 5 HEPES, 2 CaCl₂, and 2 MgATP (pH adjusted with CsOH to 7.3; 280–290 mOsm). Recordings were made using an Axopatch 200 amplifier (Axon Instruments) and started 5–10 min after a stable whole cell access was obtained. Recordings were low-pass filtered at 2 kHz with an eight-pole Bessel filter and sampled at 4 kHz on an ATARI TT030 computer using custom-made interface and software.

**Evoked mIPSCs**

In four slices (from 1 control and 3 kindled animals) also IPSCs evoked by electrical stimulation were recorded. In these experiments a second pipette (3–4 MΩ) was placed onto the surface of a pyramidal cell close to the cell of which recordings were made. A short current pulse (200 μs of 200–500 μA) was injected through the second pipette. With this pipette we searched to stimulate an interneuron that evoked an IPSC in the recorded pyramidal cell. Extracellular medium used in these latter experiments contained no TTX, only CNQX and 7-chlorotriokinurenic acid. In two of the four cells (both kindled), we succeeded in washing in the TTX-containing medium after the evoked experiment and also recorded mIPSCs from the same cells.

**Detection of mIPSCs**

Custom-made software was used to detect events off-line by comparing their waveform with that of a template. The template was constructed by averaging 100 large mIPSCs that were selected by eye. After removing the DC level just before the event, the precise timing of the events was determined by calculating the least-square error of the fit of the scaled template to the signal for each successive time point. Within a time window in which the error was below a threshold and the amplitude >13 pA, the local minimum of this error was taken as the moment of occurrence. In this way, detection of events with different amplitudes was objective, automatic, and comparable. The same template could be used in all cells, because templates constructed from different cells resulted in the same set of detected events. This detection method based on the combination of amplitude and mIPSC waveform rather than on amplitude alone proved to be less dependent of the noise level and quite robust. Hardly any improvement was seen after using additional filtering.

In both the kindled and the control group <1% of the total number of events were overlapping (time between 2 succeeding events <15 ms). Because the properties of these events will be distorted by the overlap, we only selected the event that had the best match with the template, the other was disregarded.

**Analysis of mIPSCs**

In the template matching described in the preceding text, we purposely used a low detection level. In the second phase of the analysis, an additional set of criteria was used to select those events that could reasonably be called mIPSCs. In this phase ~50% of the initially detected events were rejected, most of which had very low amplitudes.

The amplitude, rise time, and decay time constants were calculated for all events that matched the template. The sampling of the signal at 4 kHz and the noise level made it difficult to calculate fast rise times (<1 ms) with high accuracy. The rise time was defined as the time interval between the last data point with a value <20% and the first that was >80% of the peak value. The (20–80%) rise time determined in this way was overestimated by 0.5 ms at most.

The decay phase of each event was fitted with an exponential function characterized by a single time constant. Events were only considered to be mIPSCs and accepted for further analysis if the fitted decay time constant was between 5 and 250 ms and if the coefficient of determination (r²) of the least squares fit was >0.33. Fitting the decay of the current with a biexponential function hardly improved the fit, indicating that these mIPSCs have a mono-exponential decay.

For the largest mIPSCs (>75 pA), we also used nonstationary noise analysis. We scaled the mIPSCs to the mean mIPSC of the same cell.
and then plotted the variance $\sigma^2$ of the current against the mean current $I$, both calculated in bins of 2 ms. The relation between $\sigma^2$ and $I$ could be fitted with the following equation (De Koninck and Mody 1994; Sigworth 1980)

$$\sigma^2 = iI - \frac{I^2}{N}$$

resulting in an estimate of the number of postsynaptic receptors $N$ and the unitary current $i$, which can be translated to the single channel conductance $\gamma$ if the driving force is known.

**Statistics**

Differences in the mean values of mIPSC properties between the kindled and control groups were tested statistically using the Student’s $t$-test and the nonparametric Mann-Whitney test. Differences in variance were tested using the $F$ test. All data were tested per rat ($n = 8$ and $n = 11$ for kindled and control group) as well as per cell ($n = 22$ for both kindled and controls). Differences were accepted if both approaches lead to a similar conclusion and $P < 0.05$ was used to indicate a significant difference.

Distributions were compared between groups with Kolmogorov-Smirnov statistics. Possible correlations were tested with the Spearman rank-order test and with Pearson’s correlation coefficient. The first gave a good estimation of the significance of the correlation, whereas the latter gave an estimation of the strength of the correlation.

**RESULTS**

**Kindling**

During the kindling procedure field potentials in the stratum radiatum of the CA1 area were recorded in each rat. Paired-pulse stimuli were given at the same stimulation electrodes on the Schaffer collaterals through which the kindling tetani were applied. The response was quantified as the mean ratio between the minimum amplitude of the first and the second negative field potential recorded in stratum radiatum (Fig. 1). The paired-pulse ratio gradually increased from 0.9 ± 0.1 (inhibition; $n = 8$) before kindling to a value of 1.2 ± 0.1 (facilitation) after the rats had received 22 tetani. Also the shape of the field potential changed: over the same period the amplitude measured at 17 ms after the first stimulus changed from a positive overshoot (0.14 ± 0.08 mV) to a negative amplitude (−0.4 ± 0.1 mV; see Fig. 1). This reduction in paired pulse inhibition and broadening of the field potential after kindling epileptogenesis confirmed previous observations and has been interpreted as a reduced inhibitory drive (Kampfhus et al. 1988; Zhao and Leung 1993).

**mIPSCs**

All currents were recorded at room temperature from CA1 pyramidal cells, which were clamped at a membrane potential of −70 mV. During experiments, slices were perfused with ACSF that contained 7 mM KCl to increase mIPSC frequency. The input resistance of the cells of kindled and control rats were not different and in the range of 70–100 MΩ. Recordings were accepted for analysis if they were stable, that is, if the amplitude and frequency of the mIPSCs did not significantly differ during the first and the second half of the recording period. In total recordings of 22 cells from 8 kindled rats and of 22 cells from 11 control rats were performed during a time period of ~20 min per cell and contained in each group in total

**Fast and slow mIPSCs**

The most important condition that the detected events had to meet before being accepted as mIPSCs was that the decay phase was well fitted by an exponential function with a time constant between 5 and 250 ms. No additional restrictions on the rise time were necessary because the template matching already emphasized on realistic rise times. Nevertheless there was a considerable variance in the rise times of mIPSCs. The rise time distribution of each cell showed a large peak at fast rise times and a broad tail of slower rising mIPSCs (Fig. 2B). On the basis of this rise time distribution, we distinguished two groups of mIPSCs with rise times smaller and larger than 2.8 ms, which in the following we will refer to as fast and slow mIPSCs. The precise threshold of 2.8 ms for the distinction is not critical for the analysis to follow. It was an optimal separation based on a fit of the distribution with two Gaussians. Fast mIPSCs had significantly larger amplitudes than slow mIPSCs of the same cell. The decay time constants of slow rising mIPSCs tended to be somewhat larger than that of fast mIPSCs, but this difference did not reach significance (details are given in the following text).

In Fig. 2C, the means constructed by averaging 300 fast and 300 slow mIPSCs of the cell in Fig. 2B are shown. The mIPSCs were aligned (during the detection procedure) at the time point
halfway the rising phase. Of all analyzed mIPSCs ~70% were fast and 30% were slow mIPSCs. The mean rise time of slow mIPSCs was in agreement with the rise time of the mean of the same slow mIPSCs, indicating that the slow kinetics are not due to a large jitter in the detection of the mIPSCs. This was confirmed by the fact that the mean amplitude also came close to the amplitude of the mean, implying that the shapes of the mean currents as given in Fig. 2 to the amplitude of the mean, implying that the shapes of the mIPSCs was in agreement with the rise time of the mean of the slow mIPSCs, indicating that the slow kinetics are not evident in the calculated means, are discussed later.

From Table 1 it is clear that slow mIPSCs had significantly smaller amplitudes than fast mIPSCs. Fast mIPSCs had a significantly larger variance in amplitude and a smaller variance in rise time compared with slow mIPSCs. These variances were not different in the control and kindled groups (F test). Decay time constants were similar for both mIPSC types in kindled and controls and showed a large variance in all groups. The fraction of fast and slow mIPSCs per cell was not different in the kindled and control groups; from the total number of mIPSCs, 27 ± 2% (kindled) or 32 ± 3% (control) were slow. The amplitude and kinetics of the mIPSCs in this study are in good agreement with previous reports (Hájos and Mody 1997; Jarolimek and Misgeld 1997; Katchman et al. 1994; Lupica 1995).

ANOVA of the animal data showed that within the kindled and control groups the variance between and within the data per animal was not different for all parameters of Table 1. This indicates that the observed variance in mIPSC parameters

**TABLE 1. Parameters of fast and slow mIPSCs in kindled and control cells**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Kindled</th>
<th>Significance</th>
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<tbody>
<tr>
<td><strong>Fast mIPSCs</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Amplitude, pA</td>
<td>34.8 ± 1.1 (15)</td>
<td>35.4 ± 1.1 (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Rise time, ms</td>
<td>1.80 ± 0.01 (4)</td>
<td>1.79 ± 0.02 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Decay time constant, ms</td>
<td>24.8 ± 0.9 (18)</td>
<td>25.8 ± 0.7 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>0.63 ± 0.07 (51)</td>
<td>0.65 ± 0.10 (67)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Slow mIPSCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, pA</td>
<td>26.2 ± 0.7 (12)</td>
<td>26.2 ± 0.4 (7)</td>
<td>NS*</td>
</tr>
<tr>
<td>Rise time, ms</td>
<td>5.9 ± 0.2 (12)</td>
<td>5.6 ± 0.1 (10)</td>
<td>NS**</td>
</tr>
<tr>
<td>Decay time constant, ms</td>
<td>26.4 ± 0.7 (12)</td>
<td>26.8 ± 0.8 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>0.37 ± 0.04 (48)</td>
<td>0.35 ± 0.04 (49)</td>
<td>NS</td>
</tr>
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</table>

All values are means ± SD followed by the coefficient of variation (CV = 100% × SD/mean) in parentheses. Differences in mean between the kindled and control groups were tested with Student’s t-test and Mann-Whitney test, differences in variance were tested with the F test. None of the differences were significant (NS = nonsignificant difference) whether they were calculated per cell or per animal. n = 22 cells in both kindled and control group. * F test: P < 0.05; due to one control cell. ** t-test and Mann–Whitney test: P < 0.05 only when calculated per animal, not per cell.
were (determined by the 1st method) in the kindled and control groups. Both methods gave similar results. The mean mIPSC frequency was determined per 250 mIPSCs from that cell. A cell was determined as the mean of all frequencies determined by a Poisson process. The mean frequency of mIPSCs in the expected distribution for independently occurring events generated by a Poisson process. The mean frequency of mIPSCs in a cell was determined as the mean of all frequencies determined per 250 mIPSCs from that cell.

We also calculated the mIPSC frequency by determining the number of mIPSCs observed during every 100 s of recording. Both methods gave similar results. The mean mIPSC frequency (determined by the 1st method) in the kindled and control groups were (means ± SE from averaging over all cells): 0.65 ± 0.10 versus 0.63 ± 0.07 Hz for fast and 0.35 ± 0.04 versus 0.37 ± 0.04 Hz for slow mIPSCs, respectively (Table 1). Differences between kindled and control did not reach significance. The probability of occurrence of a fast or a slow mIPSC did not depend on the type of mIPSC preceding it, indicating that fast and slow mIPSCs occurred independently.

The mean frequency varied much between individual cells of the same group. The coefficients of variation (100% * SD/mean) were 67 and 49% in the kindled group and 51 and 48% in the control group. The mean mIPSC frequencies showed that this variance originated from differences between cells rather than from the variance of the individual cells.

Distributions of amplitude, rise time, and decay time constant

As mentioned in the preceding text, equal mean values for variables describing the mIPSC for the kindled and control groups do not exclude different distributions of the individual values. The types of morphological changes of the underlying synapses that have been suggested (Edwards 1995) indeed predict only subtle effects on such distributions. They even could be masked partially if not all interneurons and their synapses are affected. Such changes could nevertheless have important consequences for the efficacy of the inhibitory input. Therefore distributions of the individual mIPSC amplitudes, of the rise times, and of the decay time constants were constructed for each cell.

In Fig. 4 typical examples of the amplitude distribution of fast and slow mIPSCs of a kindled and a control cell are shown. All distributions show a peak at a value smaller than the mean amplitude and are skewed toward larger values as was reported previously (Edwards 1995; Edwards et al. 1990). Notice the broader amplitude distribution of the fast mIPSCs compared with that of the slow mIPSCs. Also the distributions of the rise time (Fig. 2B) and of the decay time constant (data not shown) were skewed toward the right.

It was possible to fit the amplitude distributions with functions previously derived by Jonas (Jonas et al. 1993) and Bekkers (Bekkers et al. 1990). However, the quality of the fit was not very high and the functions did not add sufficient explanatory power to justify further evaluation.

Large mIPSCs

The cumulative amplitude distribution gives the fraction of mIPSCs with amplitudes below a certain value. In Fig. 6A, the cumulative amplitude distributions for the kindled and control mIPSCs (pooled from all cells) are shown. The cumulative amplitude distributions show a small but highly significant difference between the kindled and the control group (Kolmogorov-Smirnov test, P < 0.0001). Notice the crossing of the two curves in Fig. 6A at ~40 pA. In the kindled group, only 3% of all mIPSC have amplitudes >75 pA, which is a significantly smaller fraction than in the control group (5%). For every given amplitude, the number of mIPSCs in both experimental groups that have larger amplitudes can be calculated. The ratio of these numbers is plotted as a function of amplitude in Fig. 6B. This graph shows that the reduction in the kindled group is systematic for all mIPSCs with amplitudes >~60 pA. The reduction in large amplitude mIPSCs also can be expressed in the frequency of occurrence. For example, mIPSCs with amplitudes >75 pA occurred with a frequency of 0.042 Hz in the control group, whereas this was only 0.027 Hz in the kindled group. The frequencies were calculated by dividing the total number of observed large mIPSCs (1,094 for the controls and 727 for the kindled) by the total recording time for both groups. The tails of the amplitude distributions are shown in detail in Fig. 6C. The high significance of the difference in amplitude distribution did not depend on the part of the distribution in the small amplitude range, which was the part most affected by our detection method. Hence, the precise separation between small and large mIPSCs did not influence this conclusion.

The distributions of mIPSC rise times in the kindled and

![Graph showing the distribution of time intervals between successive mIPSCs](http://jn.physiology.org/ by 10.220.33.4 on November 7, 2016)
control group showed a small significant difference in the slow rise time region (rise time >5 ms; Fig. 5C). The distributions of the decay time constants were not different in the kindled and control groups (Fig. 5, A and B). The slow mIPSCs showed a higher percentage of large decay time constants compared with the fast mIPSCs.

Relations between amplitude, rise time, and decay time constants

The different properties of the mIPSCs within one cell are to some extent related (Fig. 7). No significant correlations were found between amplitudes, rise times and decay time constants of individual mIPSCs, neither in kindled nor in control cells. However, the graphs clearly illustrate that the mIPSCs are not drawn from a homogeneous group with normally distributed parameter values. The mIPSCs with the largest amplitude (>75 pA) form a group with significantly faster rise and decay times than the mean. The very slow mIPSCs (rise time >5 ms) have significantly smaller amplitudes. The mIPSCs with the fastest rise times most likely originate from sites with the least effect of dendritic filtering. In this group of mIPSCs, however, a large variation in amplitude (Fig. 7A) and decay time constant (Fig. 7B) was observed even within the same recording. This indicates that the variation is intrinsic.

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**FIG. 4.** Amplitude distributions of fast (top) and slow mIPSCs (bottom) from a kindled (A) and control (B) cell (binwidth = 3 pA). Peaks are normalized to 1 to allow a good comparison. All distributions are skewed toward higher values. Notice the larger variance in amplitude of the fast mIPSCs compared with the slow mIPSCs.

**FIG. 5.** A: cumulative distributions of decay time constants of mIPSCs from the pooled kindled (dashed) and control (solid lines) groups were not significantly different. Distributions of fast mIPSCs are plotted with thick lines, those of the slow mIPSCs with thin lines. B: ratio of the number of mIPSCs with decay time constants larger than a certain value (ordinate) in the kindled and control group illustrating no difference in the distributions of the decay time constants. C: ratio of the number of mIPSCs with rise times larger than a certain value (ordinate) in the kindled and control group illustrating a small significant difference in the slow rise time region (compare Fig. 6, B and C, with Fig. 5B).
Noise analysis

We applied nonstationary noise analysis to the group of the largest mIPSCs (amplitude >75 pA) to estimate the single channel conductance and number of postsynaptic GABA$_A$ receptors that contributed to the event (Fig. 8). There were 727 large mIPSCs from kindled and 1,094 from controls. The mean single-channel conductance was 30 ± 9 pS in the kindled and 29 ± 5 pS in the control group, which is in good agreement with other reports about the GABA-mediated chloride channels (De Koninck and Mody 1994; Edwards et al. 1990; Grudt and Henderson 1998; Puopolo and Belluzzi 1998). The estimated mean number of postsynaptic receptors was 87 ± 6 and 99 ± 6 for kindled and controls, respectively. None of these differences reached significance.

Evoked IPSCs

In four cells, we recorded IPSCs that were evoked by a current injection from a large-tip glass electrode, filled with ACSF and positioned on the surface of a nearby pyramidal neuron. The stimulation most likely activated axons from inhibitory neurons that project to the recorded cell (no extracellular TTX). Stimulus intensity varied between 200 and 500 μA. The evoked IPSCs show a small delay after the stimulus (Fig. 9). Failures were observed at all stimulus intensities, but as expected most occurred at low intensity. The amplitudes, rise time, and decay time constants were determined of all evoked IPSCs in the same way as was done for the mIPSCs. The mean rise time and decay time constant were 1.7 ± 0.1 and 16.4 ± 1.0 ms ($n = 104$). The small number of successful evoked IPSC recordings did not allow a conclusive comparison between kindled and controls. Recordings of evoked and miniature IPSCs obtained from the same cell were, however, useful to indicate the good agreement between the kinetics of the evoked IPSCs and the scaled mean of the fast mIPSCs (Fig. 9B). The mean amplitude of evoked IPSCs was larger than that of the mIPSCs: 57 ± 2 pA at a low stimulus intensity of 200 μA ($n = 19$, excluding failures).

DISCUSSION

The enhanced excitability characteristic for epilepsy is brought about by a shift in balance from inhibitory to excitatory drive. A multitude of mechanisms can be involved starting from a direct enhancement of excitatory transmission (Köhr et al. 1993; Kraus et al. 1994) or reduction in inhibition (Kamphuis et al. 1988; Zhao and Leung 1993) up to quite specific changes in the excitability of specific cells (Faas et al. 1996; Vreugdenhil and Wadman 1992; Vreugdenhil et al. 1998) or the functional loss of strategically important cell classes (Kamphuis et al. 1989; Sloviter 1991). Previous field potential recordings (confirmed in the present study) showed a gradual reduction in functional inhibition in the CA1 network during kindling epileptogenesis (Kamphuis et al. 1988). This effect is maximal immediately after the kindling stimulations, but partial recovery has been observed in the long-term animals (Zhao and Leung 1993).

A special group of large mIPSCs was distinguished in the total population of mIPSCs recorded in CA1 pyramidal cells. This group was distinct in amplitude, and it showed significantly faster kinetics than the mean population event. In long-term kindled animals a 40–50% reduction in the number of mIPSCs in this group was found. Although these large mIPSCs were only a small fraction of the total number of mIPSCs, the specific reduction seen in this group was responsible for a highly significant difference in the amplitude distribution of mIPSCs after kindling.

The mean mIPSC amplitude and frequency and the total number of fast and slow mIPSCs were not different in the kindled and the control group. The characteristic properties of the individual mIPSCs (kinetics for all mIPSCs, single-channel conductance and number of postsynaptic channels for only mIPSCs with amplitudes >75 pA) reflect the properties of the GABA$_A$ receptors involved. These were not different in the two experimental groups.
Fast and slow mIPSCs

We found fast and slow mIPSCs in the CA1 pyramidal neurons. The fast mIPSCs could represent currents originating from synapses on the soma and proximal dendrites, whereas the slow mIPSCs could be generated in synapses located more distally on the dendrites. Linear correlations between kinetics and amplitudes of mIPSCs might be obscured by the large intrinsic variance in amplitude and decay time constants. Different interneuron classes could evoke IPSCs with specific kinetics (Ouardouz and Lacaille 1997) and differences in pharmacological properties between fast and slow mIPSCs cannot be ruled out (Banks et al. 1998; Pearce 1993). The properties of fast and slow mIPSCs overlap, but the precise threshold level used for qualification hardly affected the conclusions drawn here. Several factors could underestimate the ratio between slow and fast mIPSCs. Small mIPSCs from dendritic synapses are filtered and may not be detected in the soma. The noise level will also bias against the small amplitude mIPSCs. As a consequence, the observed ratio should not be quantitatively translated to the distribution of synapses over the cell surface.

Postsynaptic GABA<sub>A</sub> receptors

Kindling epileptogenesis in CA1 is associated with a decrease in binding density of the GABA agonist muscimol (Titulaer et al. 1994). This decrease could reflect a change in receptor affinity, it could indicate a reduction in GABA receptor density per synapse or a reduction in the absolute number of GABAergic synapses. The latter two possibilities should lead to differences in mIPSC amplitude and frequency after kindling, none of which were found. If GABA receptors operate in a saturated mode (Edwards et al. 1990; Faber et al. 1992; Jonas et al. 1993), changes in receptor affinity will not be reflected in mIPSC properties. However, reduced affinity should decrease the amplitude of mIPSCs that are mediated by unsaturated receptors as has been suggested for the large amplitude mIPSCs (Nusser et al. 1997).

The noise in the decay of a mIPSC reflects gating of the postsynaptic GABA<sub>A</sub> receptors (Borst et al. 1994; De Koninck and Mody 1994; Sigworth 1980). The number of receptors and their single-channel conductance can be estimated if channel noise dominates. For the largest mIPSCs, we obtained numbers in agreement with previously reported values for GABA<sub>A</sub>
The consequences of kindling induced changes at the synapse level also have to be considered at the network level. The relation between the mIPSCs caused by spontaneous release of vesicles and functional inhibitory synapses in the intact network is not evident (Faber et al. 1998). In the cells in which we were able to record both miniature and evoked IPSCs, we observed a striking similarity between their kinetics. This at least suggests that the evoked IPSCs consisted of the synchronous activation of synapses from the same type that produced the mIPSCs.

If linked to functional synapses, the reduction in mIPSCs reflects a loss of inhibitory innervation. The group of mIPSCs with high amplitudes and fast kinetics most likely originates from the soma. Because the somatic inhibitory synapses are the most effective, even a relative small reduction in their number could lead to a noticeable impairment of inhibition. The fact that also the fraction of very slow mIPSCs (rise time >5 ms) is smaller after kindling could indicate that the loss of synapses is not restricted to the soma. Previous immunocytochemical work (Kamphuis et al. 1989) has shown that 50% of the GABAAergic interneurons that do not contain parvalbumin have disappeared after kindling epileptogenesis. Whether the synapses of these interneurons are responsible for the large amplitude mIPSCs needs to be determined.

Functional rewiring of the local circuit with a change in function of specific neurons has been observed after epileptogenesis and other challenging conditions. Bragin reported a reduced activity of the interneurons in the CA1 area during an epileptic seizure, indicative of specific participation of different classes of neurons in epileptic activity (Bragin et al. 1997). Long-term stimulation that induces epilepsy leads to the loss of hilar neurons in the dentate gyrus and so removes the excitatory input from interneurons which then become dormant (Sloviter 1991). This phenomenon was not observed in the self-sustained limbic status epilepticus model (Rempe et al. 1997), but after anoxia functionally disconnected interneurons also have been reported (Khazipov et al. 1995). Denervated interneurons result in normal GABAAergic innervation of the principal neurons, which due to the lack of excitatory input on the interneurons will never function. But the opposite situation also exists. Unchanged GABAAergic innervation of principal neurons will be more effective if the interneurons that drive it are innervated more effectively or highly synchronized. Sprouting that occurs heavily in the dentate gyrus (Cavazos et al. 1994) could compensate for the loss of cells and reduction in connectivity, but such compensation would most likely result in a higher degree of synchronization. In the CA1 area sprouting is more disputed (Perez et al. 1996), but also much harder to detect.

**Comparison with dentate gyrus**

The observations in the dentate gyrus after kindling epileptogenesis are almost opposite to the ones that we report for the CA1 area. In the dentate gyrus, the mean mIPSC amplitude was increased (Otis et al. 1994), and this change recently was found to be linked to an increase in the number of postsynaptic GABAA receptors (Nusser et al. 1998). These findings are consistent with an overall enhancement of the GABAAergic inhibition in the dentate gyrus as has been reported (Kamphuis et al. 1992; Oliver and Miller 1985). They are, however, hard to link causally to the emergence of epileptic activity (but see Buhl et al. 1996). In the CA1 area, the changes in mIPSCs and

**Synapse morphology**

A hypothesis proposed by Edwards predicted that plasticity of synapses induces morphological changes reflected in the skewness of the amplitude distribution (Edwards 1995). Large-amplitude miniature postsynaptic currents can either originate from synapses with a large number of receptors or they reflect the synchronous release of vesicles from several active zones (Edwards 1995). Consequently, the reduction in large-amplitude mIPSCs in kindled animals should show up morphologically as a reduction in the number of synapses with a large postsynaptic grid or multiple active zones. GABAAergic synapses with several separate active zones within one synapse have been reported (Nusser et al. 1997; Peters et al. 1990). An increase in the number of perforated synapses and an increase in synaptic area of GABAAergic synapses were shown previously in dentate gyrus after kindling epileptogenesis (Geinisman et al. 1990; Nusser et al. 1998).

**Network function**

The consequences of kindling induced changes at the synapse level also have to be considered at the network level. The

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**Fig. 9.** Evoked IPSCs. A: IPSCs were evoked by current injection (300–500 μA) onto the surface of an adjacent pyramidal cell. Some stimuli failed to induce an IPSC (not further quantified). B: mean of 100 fast mIPSCs (thin line) recorded from the same cell is scaled to the peak of an evoked IPSC and aligned, illustrating very similar kinetics of evoked and miniature IPSCs.
field potential inhibition are also consistent, but in the opposite direction. Here they could underlie the emergence of epilepsy. These findings indicate that different brain areas can react differently on the establishment of an epileptic focus.

A reduction in inhibitory drive in the CA1 network after kindling epileptogenesis will involve changes in cell properties and in their functional connectivity. Because the epileptic network is properly functioning most of the time, the changes observed after kindling are expected to be subtle. At the cell membrane level, many changes in properties or abundance of calcium and sodium channels have been reported (Vreugdenhil and Wadman 1992; Vreugdenhil et al. 1998), but these changes need not be the same in all cells of the network. The transfer of a local network can be changed in even more ways because it comprises the changes in all cells and their connectivity. Synapses can adapt their strength depending on the activity in pre- and postsynaptic cells (Davis and Goodman 1998; Turrigiano et al. 1998).

In the CA1 region, kindling epileptogenesis induces a shift in the balance between inhibition and excitation. In pyramidal cells, membrane excitability is enhanced, whereas in the network, inhibition is reduced. Our new data suggest that the loss of specific somatic synapses or of the projecting interneurons could be an important factor. The net result of all mechanisms is a lower threshold for seizure activity. In particular in the kindling model, the network is stable for most of the time; this suggests that the changes are at least partly compensated. The most intriguing mechanism, the one that defines the long-term balance between excitation and inhibition, still needs to be determined.

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