Changes in mIPSCs and sIPSCs after Kainate Treatment: Evidence for Loss of Inhibitory Input to Dentate Granule Cells and Possible Compensatory Responses

(Running title: Dentate inhibition and epilepsy)

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ABSTRACT

How inhibition is altered after status epilepticus and the role of inhibition during epileptogenesis remain unsettled issues. The present study examined acute (4-7 days) and chronic (>3 months) changes of GABA_A-receptor-mediated inhibitory synaptic input to dentate granule cells after kainate-induced status epilepticus. Whole-cell patch-clamp techniques were used to record spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX) and DL-2-amino-5-phosphonopentanoic acid (AP-5) to block glutamatergic excitatory synaptic transmission. In both groups, mean sIPSC frequency of dentate granule cells from the saline- and kainate-treated rats was not significantly different. However, mIPSC frequency from the kainate-treated rats of both groups was about 30% lower than that of the respective saline controls. The mean amplitude of sIPSCs and mIPSCs from kainate-treated rats was not reduced in either the acute or chronic groups. The mean 10-90% rise time of IPSCs was not altered in kainate-treated rats, but the decay time constant was slightly longer than in controls, and the charge transfer 4-7 days after kainate treatment was significantly larger. The similar reduction of mIPSC frequency (i.e., ~30%) in the two groups of kainate-treated rats suggests a decreased inhibitory input to dentate granule cells (presumably due to a partial loss of inhibitory interneurons that innervate them), without recovery during epileptogenesis. The lack of effect on sIPSC frequency and the decreased mIPSC frequency in both groups suggests a possible compensatory increase in firing rate of interneurons, which may involve a hypothetical reduction of inhibitory input to the remaining interneurons.

Key words: epileptogenesis; inhibition; GABA; status epilepticus; whole-cell recording
INTRODUCTION

Epilepsy is a disorder characterized by the occurrence of spontaneous recurrent seizures, which are prolonged and synchronized neuronal discharges. The abnormal activity may result from enhanced excitation, reduced inhibition, or both. The hippocampal formation is regarded as a common epileptic focus of temporal lobe epilepsy, one of the most common and intractable types of epilepsy (Engel, 1989). The dentate gyrus has attracted numerous studies because it undergoes consistent pathological changes during epileptogenesis, such as loss of inhibitory neurons (Davenport et al., 1990a; Obenaus et al., 1993) and sprouting of excitatory axons (e.g., Tauck and Nadler, 1985).

One major issue is how inhibition is altered during epileptogenesis. Many studies have revealed significant neuronal degeneration, including loss of interneurons, in both human epileptic tissue (Babb et al., 1984; de Lanerolle et al., 1989; Mathern et al., 1995a, b) and in animal models of epilepsy (Buckmaster and Dudek, 1997a; Buckmaster and Jongen-Relo, 1999; Davenport et al., 1990a; Dinocourt et al., 2003; Houser and Esclapez, 1996; Kobayashi and Buckmaster 2003; Obenaus et al., 1993; Sayin et al, 2003). Despite the anatomical evidence of interneuron loss, electrophysiological studies have reported relatively normal or enhanced inhibition (Buckmaster and Dudek, 1997b, 1999; Buhl et al., 1996; Esclapez et al., 1997; Haas et al., 1996; Wilson et al., 1998; Wu and Leung, 2001), in addition to evidence for decreased inhibition (Kobayashi and Buckmaster, 2003; Sloviter, 1987, 1991; Williamson et al., 1995, 1999) in both human epileptic tissue and animal models of epilepsy. Therefore, whether the inhibitory system is impaired in an epileptic brain and its role in epileptogenesis remain an unsettled issue.
The present study was designed to further address this issue in rats with kainate-induced epilepsy. Using spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) of granule cells as functional indicators of interneuron activity and viability, we aimed to address the following questions: Does kainate-induced status epilepticus cause an immediate reduction of GABA_A-receptor-mediated inhibitory synaptic input to granule cells (i.e., damage to or loss of dentate interneurons)? If so, does the damage progress or recover during the course of epileptogenesis?

**METHODS**

*Animal treatment*

All procedures used in this study were approved by the Colorado State University Animal Care and Use Committee. As described previously (Hellier et al., 1998; Smith and Dudek, 2001; Wuarin and Dudek, 2001), male Sprague-Dawley rats (Harlan, ~175 g) received multiple kainate injections (5 mg/kg, intraperitoneal) at an interval of 1 h, until they had recurring class IV/V seizures (Ben-Ari, 1985; Racine, 1972) for \( \geq 3 \) h (i.e., status epilepticus). Control rats received saline injections in parallel with kainate-treated rats. After kainate treatment, rats were monitored for 1-2 h/day, 3-5 days/week to estimate the frequency and severity of spontaneous motor seizures.

*Slice preparation*

The acute group was comprised of rats studied 4 to 7 days after kainate or saline treatment, and the chronic group included rats studied >3 months after treatment, when the kainate-treated rats had been observed to have spontaneous seizures. Rats were coded
and obtained by another person so the experimenter was blind to the type of treatment (saline or kainate). Rats were anesthetized with halothane and decapitated with a guillotine. Their brains were quickly dissected out and placed in partially frozen oxygenated artificial cerebrospinal fluid (ACSF) composed of (in mM): 124 NaCl, 3 KCl, 26 NaHCO$_3$, 1.4 NaH$_2$PO$_4$, 1.3 CaCl$_2$, 1.3 MgSO$_4$ and 11 glucose. Hippocampal slices (300 µm thick) were cut parallel to the base of the brain with a vibroslicer (Lancer series 1000, Vibratome, St. Louis, MO), mostly from the temporal part of the hippocampus. Slices were incubated in a submerged chamber at 32–34 °C for 2 h to recover.

*Recording procedure and data acquisition*

For electrophysiological experiments, slices were submerged in a recording chamber and perfused with oxygenated ACSF. Whole-cell patch-clamp recordings were conducted at room temperature using glass pipettes that were pulled from borosilicate glass capillaries (OD 1.65 mm, ID 1.2 mm, Garner Glass, Claremont, CA) with a P-87 Flaming-Brown puller (Sutter Instruments, Novato, CA, U.S.). The intracellular solution was comprised of (in mM): 130 CsCl, 1 NaCl, 5 EGTA, 10 HEPES, 1 MgCl$_2$, 1 CaCl$_2$, 5 QX314, 2 ATP and 5 biocytin. The pH was adjusted to 7.2 with CsOH. When filled with this solution, the patch pipettes had resistances of 2-3 MΩ. A calculated liquid junction between pipette solution and bath solution (ACSF) at 20 °C was +2.9 mV (Clampex software, Axon instruments, Foster City, CA) and was not corrected. The series resistance in the whole-cell configuration was 6-10 MΩ, estimated from the amplitude of the initial capacitive transient in response to a 5 mV, 30 ms hyperpolarizing pulse. Series resistance was uncompensated and monitored during each experiment. Only data without changes in
series resistance during the experiment were included in this study. The actual input resistance and resting membrane potential could not be determined because of the presence of intracellular Cs\(^+\), which blocks all K\(^+\) channels. The chloride equilibrium potential (based on the Nernst equation) was +1 mV under the present conditions, and membrane potential was clamped at -70 mV, so the IPSCs were inward currents. Spontaneous EPSCs were excluded from recordings by adding glutamate receptor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX, 50 µM) and DL-2-amino-5-phosphonopentanoic acid (AP-5, 50 µM); therefore, all of the recorded inward currents were IPSCs. Miniature IPSCs (mIPSCs) were obtained by adding 2 µM tetrodotoxin (TTX). Each new batch of TTX solution was tested to show that it effectively eliminated action potentials. Bicuculline (30 µM) was added at the end of each experiment to verify that GABA\(_A\) receptors mediated all of the recorded currents. All signals were amplified with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA), low-pass filtered at 2 kHz and sampled at 10 kHz, and recorded with pClamp 8.0 software (Clampex, Axon Instruments, Foster City, CA) through a Digidata-1320A digitizer (Axon Instruments, Foster City, CA).

**Data analysis and statistics**

The pClamp 8.0 (Clampfit, Axon Instruments, Foster city, CA, U.S.) and MiniAnalysis 5.0 programs (Synaptosoft, Inc., Leonia, NJ, U.S.) were used for qualitative and quantitative data analysis. The IPSCs were detected using MiniAnalysis software. The threshold value for detecting IPSCs was set as twice the baseline noise (root-mean-square, RMS) and the IPSCs detected by the software were visually checked to minimize
errors. The mean noise level (RMS) for the control and kainate groups of 4-7 days and >3
months after treatment was: 2.1 ± 0.1, 2.3 ± 0.1, 2.6 ± 0.2 and 2.7 ± 0.1 pA, respectively.
A 3-min epoch of recording before and after TTX per cell was used for analyzing sIPSCs
and mIPSCs, respectively. To minimize potential sampling bias, a maximum of two cells
per animal were recorded, and a fixed number of sIPSCs and mIPSCs from each neuron
(i.e., the first 200 sIPSCs and the first 100 mIPSCs) was pooled for constructing
histograms for amplitude and interval distributions, and for calculating cumulative
probability. Values of all IPSC intervals were logarithmically transformed in the
histograms. The 10-90% rise time, decay time constant, and area (pA×ms) of IPSCs was
automatically measured using MiniAnalysis software (Synaptosoft, Inc., Leonia, NJ,
U.S.). The charge transfer of IPSCs presented in following sections was represented by
the area of the currents. The Kolmogorov-Smirnov (KS) two-sample, two-tailed test was
used to compare the cumulative probability of sIPSC and mIPSC intervals between
control and epileptic groups. The mean frequency, amplitude, 10-90% rise time, decay
time constant and charge transfer for sIPSCs and mIPSCs were averaged across neurons
(i.e., n=cells, not events). The two-tailed Student’s t-test and the Analysis of Variance
(ANOVA) were used for comparisons between two groups and among multiple groups,
respectively. Data are expressed as means ± SE, and α=0.05 in all tests.
RESULTS

IPSCs recorded from 73 dentate granule cells were analyzed in this study, including 36 neurons from rats 4-7 days after kainate or saline treatment (i.e., acute group) and 37 neurons from rats >3 months after treatment (i.e., chronic group).

*sIPSCs and mIPSCs 4-7 days after treatment*

One functional approach to examine whether GABAergic interneurons that project to dentate granule cells were immediately injured or otherwise altered shortly after kainate-induced status epilepticus is to measure the change of IPSCs in dentate granule cells. The sIPSCs and mIPSCs were recorded in granule cells from rats 4-7 days after kainate or saline treatment. Robust sIPSCs with a frequency up to 10 Hz and an amplitude of hundreds of picoamperes were observed in nearly all of the recorded granule cells from control rats (Fig. 1A, n=17), suggesting a strong inhibitory control of granule cells under normal physiological conditions. A similar pattern of sIPSCs was also observed in granule cells from kainate-treated rats (Fig. 1B, n=19). Quantitative analyses showed that the distribution of inter-IPSC intervals of the control and kainate groups was very similar (Fig. 2A1), and their cumulative probability was identical (Fig. 2A2, p>0.05, K-S test). These data suggested that the sIPSC frequency of granule cells 4-7 days after kainate-induced epilepticus remained the same as controls. Next, we examined whether the mIPSCs of the granule cells were changed 4-7 days after kainate treatment. The mIPSCs were obtained from the same population of recorded neurons as sIPSCs, after
addition of TTX (i.e., application of TTX eliminated large-amplitude, action potential-dependent IPSCs and left mIPSCs; Fig. 1). In contrast to the sIPSCs, most mIPSCs from both groups had amplitudes of several picoamperes to several tens of picoamperes and were comparatively infrequent (Fig. 1). Quantitative analyses of the mIPSCs revealed that the distribution of inter-mIPSC intervals of both groups fit a Gaussian distribution (Fig. 2B1), but the peak interval of the kainate group was clearly shifted to the larger side (Fig. 2B1, lower), compared with the control group (Fig. 2B1, upper, dotted line). Also, the cumulative probability of mIPSC intervals of the two groups was clearly different (Fig. 2B2, p<0.05, K-S test). These data show that the frequency of mIPSCs in the kainate-treated group was significantly lower than the mIPSC frequency from the control group, suggesting a reduced number of inhibitory input to the granule cells 4-7 days after kainate-induced state epilepticus.

sIPSCs and mIPSCs >3 months after treatment

The data above appear to indicate an immediate reduction of inhibitory inputs to dentate granule cells after status epilepticus. Possible hypothetical consequences following such an acute change are: (1) continued and progressive loss of inhibitory input, (2) recovery from the loss of interneuron input, or (3) no additional changes in interneuron input to granule cells. To address this question, we analyzed mIPSCs and sIPSCs in granule cells from rats >3 months after kainate or saline treatment. The kainate-treated rats had been observed to have developed spontaneous recurrent seizures, and the seizure rate for the last 2 weeks before they were studied ranged from 0.25 to
0.67 seizures/h, which was consistent with a previous study from our laboratory (Hellier et al., 1998). Similar to the acute groups, large and relatively frequent sIPSCs and numerous mIPSCs were also present in granule cells from both control and kainate rats >3 months after treatment (Fig. 3). The distribution of inter-sIPSC intervals was similar between the two groups (Fig. 4A1) and the cumulative distribution was identical (Fig. 4A2, p>0.05, K-S test). However, as in the acute groups, the distribution of inter-mIPSC intervals revealed a clear difference between the kainate-treated and the saline treated groups. That is, the peak mIPSC interval of the kainate group shifted to the right (Fig. 4B1, lower) compared to the controls (Fig. 4B1, upper, dotted line). The cumulative distribution of mIPSC intervals of the two groups was also different (Fig. 4B2, p<0.05, K-S test). These data showed reduced inhibitory input to granule cells in rats >3 months after kainate treatment, which suggest that the initial damage to the inhibitory neurons caused by kainate-induced status epilepticus was still present and not detectably altered at the time when spontaneous seizures had developed.

Comparison of acute and chronic changes in the frequency and amplitude of sIPSCs and mIPSCs in granule cells after kainate-induced epilepticus

The data above showed that the interval distribution (or frequency) of mIPSCs, but not sIPSCs, was significantly different for control versus kainate groups at both short (i.e., 4-7 days) and long time points (>3 months) after treatments. As expected, these differences and similarities were also seen in further analyses of the mean frequency for each group. The mean frequency of sIPSCs between the kainate and saline groups at 4-7
days or >3 months after treatment was similar (Fig. 5, 3.50 ± 0.50 vs. 3.60 ± 0.60 Hz and 2.95 ± 0.34 vs. 2.90 ± 0.33 Hz, respectively, p>0.05, unpaired t-test). The mean sIPSC frequency in the >3 month groups appeared smaller than in 4-7 day groups (~15-19% less), but the difference was not statistically significant (p=0.6, ANOVA) across all of the four groups. In contrast, the mean mIPSC frequency from rats 4-7 days and >3 months after kainate treatment was about 30% less than that in the controls (Fig. 5, 1.33 ± 0.13 vs. 1.86 ± 0.20 Hz and 1.55 ± 0.22 vs. 2.19 ± 0.21 Hz, respectively, p<0.05, unpaired t-test). The difference in mIPSC frequency between the two control groups was not significant (p>0.05, unpaired t-test). The similar reduction of the mIPSC frequency in the acute and chronic kainate groups suggests that the initial loss of inhibitory inputs to granule cells remained unchanged during the course of epileptogenesis (see DISCUSSION). In contrast to the changes in mIPSC frequency, the mIPSC amplitude from rats 4-7 days after kainate treatment was increased (Fig. 6A, 46.26 ± 2.18 vs. 30.18 ± 2.10 pA, p<0.01, unpaired t-test), and the sIPSC amplitude had a tendency towards an increase, but the difference was not statistically significant (Fig. 6A, 63.34 ± 4.76 vs. 51.89 ± 5.99 pA, p>0.05, unpaired t-test). In contrast, both sIPSC amplitude and mIPSC amplitude in rats >3 months after kainate treatment were similar to those in the controls (Fig. 6B, 50.00 ± 4.07 vs. 50.60 ± 4.80 pA and 38.96 ± 2.87 vs. 38.88 ± 4.13 pA, respectively, p>0.05, unpaired t-test).
Analyses of IPSC kinetics

The reduction of mIPSC frequency after kainate treatment supports the hypothesis that some interneurons that innervate dentate granule cells die or are damaged after status epilepticus. The next question is which interneurons are lost? One functional approach to address this question is to measure the rise time of the IPSCs, because it is thought that the interneurons that form synapses close to the somata of granule cells (i.e., peri-somatic sites) produce IPSCs with a fast rise time, whereas interneurons that form synapses relatively distal to the granule cell somata (i.e., dendritic sites) produce IPSCs with a slow rise time when the IPSCs are recorded at the soma (Kobayashi and Buckmaster, 2003; Wierenga and Wadman, 1999). In this study, the mean 10-90% rise time of the mIPSCs from rats 4-7 days after kainate treatment appeared to be slightly shorter but was not significantly different from the controls (Fig. 7A, 1.74 ± 0.17 vs. 1.95 ± 0.11 ms, p>0.05, unpaired t-test), and was not different from that of sIPSCs (Fig. 7A, p>0.05, ANOVA). Similarly, the mean 10-90% rise time of mIPSCs in rats >3 months after kainate was not significantly different from the controls or from that of sIPSCs (Fig. 7B, p>0.05, ANOVA). Were other kinetic parameters of the mIPSCs altered after kainate treatment? The decay time constant of the mIPSCs in rats from both 4-7 days and >3 months after kainate treatment appeared to be slightly but significantly longer than the controls (Fig. 8A, 18.80 ± 0.69 vs. 16.50 ± 0.69 ms, p=0.025 and 18.48 ± 0.35 ms, p=0.035, unpaired t-test). A similar change was also seen in sIPSCs (not shown). The charge transfer of mIPSCs (represented by the area of the currents) in rats 4-7 days after kainate treatment was increased (Fig. 8B, 664.58 ± 25.04 vs. 389.59 ± 20.21
pA×ms, p<0.01, unpaired t-test). In contrast, in rats >3 months after kainate treatment, the mIPSC charge transfer showed a tendency towards an increase, but was not significantly different (Fig. 8B, 566.67 ± 59.63 vs. 504.11 ± 36.53 pA×ms, p>0.05, unpaired t-test). A similar change was also seen in sIPSCs (not shown).

**DISCUSSION**

The main results of the present study are that the mIPSC frequency of dentate granule cells, but not the sIPSC frequency, was reduced 4-7 days after kainate-induced status epilepticus. A similar degree of reduction of mIPSC frequency, but not sIPSC frequency, was also present >3 months after kainate treatment when the rats had developed spontaneous recurrent seizures. The sIPSC and mIPSC amplitude at either 4-7 days or >3 months after kainate treatment was not decreased (mIPSC amplitude was actually increased at 4-7 days after treatment). The 10-90% rise time of IPSCs at both 4-7 days and >3 months after kainate treatment was not altered, but the decay time constant of IPSCs was somewhat prolonged, and the charge transfer of IPSCs was increased at 4-7 days but not >3 months after kainate treatment.

*Reduced mIPSC frequency 4-7 days and >3 months after kainate treatment*

The mIPSCs of a dentate granule cell are believed to be caused by action potential-independent release of GABA (i.e., quantal release, Edwards et al., 1990) from the presynaptic terminals of GABAergic neurons. The frequency of mIPSCs is thus
determined by the total number of presynaptic axon terminals that synapse on the postsynaptic neuron and the quantal-release probability at the individual synapses, but a decrease in mIPSC amplitude (e.g., due to decreased number of postsynaptic receptors) could also lead to an apparent reduction in mIPSC frequency (i.e., if the smallest mIPSCs were undetected). In the present study, a reduction of mIPSC frequency was observed after kainate treatment, and this reduction cannot be explained by a decrease of postsynaptic receptors because the amplitude of the mIPSCs was not reduced (mIPSC amplitude was actually increased). The reduced mIPSC frequency might have resulted from a decreased transmitter-release probability after kainate treatment (Hirsch et al., 1999), but the lack of a decrease in sIPSC frequency and amplitude in the same granule cells argues against this hypothetical mechanism. Therefore, the reduction of the mIPSC frequency most likely reflects a decrease in the number of presynaptic interneuron terminals that synapse on dentate granule cells. Hence, the observation of a ~30% reduction in mIPSC frequency in slices obtained from rats 4-7 days after kainate treatment suggests that kainate-induced status epilepticus causes an immediate but partial loss of inhibitory input to dentate granule cells, and supports studies that have shown a significant loss of hippocampal interneurons after status epilepticus using this model (Buckmaster and Dudek, 1997a), the pilocarpine model (Dinocourt et al., 2003; Kobayashi and Buckmaster, 2003), and the kindling model (Sayin et al., 2003).

The frequency of mIPSCs and the degree of reduction (i.e., ~30%) were smaller than that observed in the study of Kobayashi and Buckmaster (2003), which showed a >50% reduction 3-7 days after pilocarpine-induced status epilepticus. The possible explanations for these quantitative differences include the animal models (kainate vs. pilocarpine) and the duration of status epilepticus.
pilocarpine), recording conditions (room temperature vs. 30-31°C) and slice preparation (300 vs. 350 µm of thickness). In particular, pilocarpine treatment tends to kill more dentate interneurons than kainate treatment. The latter primarily damage the somatostatin-positive interneurons (Buckmaster and Dudek, 1997a; Buckmaster and Jongen-Relo, 1999), whereas the former injures both somatostatin- and parvabumin-positive interneurons (Kobayashi and Buckmaster, 2003), which may lead to a larger reduction in mIPSC frequency.

The degree of reduction (i.e., 30%) in mIPSC frequency observed in rats >3 months after kainate treatment was nearly identical to the reduction seen at 4-7 days. A straightforward explanation for the similar reduction in mIPSC frequency in granule cells at the acute and the chronic stages is that the initial damage to the inhibitory neurons caused by status epilepticus does not worsen or recover during the course of epileptogenesis. We cannot, however, rule out more complex possibilities. For example, a progressive loss of inhibitory inputs after the status epilepticus may be followed by partial recovery due to either axonal sprouting of the interneurons (Davenport et al., 1990b; Mathern et al., 1997) or neurogenesis (Parent et al., 1997; Scharfmann et al., 2000). Resolution of this issue in regard to these latter possibilities will require additional studies that involve more time points after the status epilepticus, in conjunction with measures of axon sprouting and neurogenesis of GABAergic neurons.

Lack of reduction in sIPSC frequency 4-7 days and >3 months after kainate treatment
Despite the reduction in mIPSC frequency in the acute and chronic kainate groups relative to controls, the sIPSC frequency remained unchanged in kainate-control comparisons at both time points. Since the sIPSC frequency is the sum of the action potential-dependent release and the release that is independent of action potentials, the lack of reduction in sIPSC frequency (while mIPSC frequency was reduced in the same granule cells) suggests a possible compensatory increase in firing rate of the remaining interneurons. It is unclear, however, what mechanisms might cause such a compensatory increase of interneuron firing. One possibility, similar to the neocortex (Bacci et al., 2003; Tamas et al., 1998), is that the dentate interneurons are connected with each other (Hajos and Mody, 1997). Therefore, some of the surviving GABAergic interneurons that project to granule cells may also lose inhibitory input from other interneurons that die or are damaged after status epilepticus. As a result, the remaining interneurons might become more active. Another possibility is that kainate-induced status epilepticus causes an increase in endogenous membrane excitability of the surviving interneurons, such that they fire more spontaneous action potentials. These hypotheses, however, remain to be tested with recordings from dentate interneurons.

In contrast to the studies that have shown a significant loss of dentate interneurons in epileptic tissues or animal models, the “dormant basket cell” hypothesis (Sloviter, 1991) proposes that the dentate interneurons lose glutamatergic excitatory synaptic input after the kainate-induced status epilepticus. The observation of a higher frequency of sIPSCs compared to mIPSCs in dentate granule cells after kainate-induced status epilepticus does not support the hypothesis that the dentate interneurons become “dormant” (Sloviter, 1991, 2003); at least some of the interneurons continue to fire.
spontaneous action potentials in this model even when glutamate-mediated transmission has been blocked. In the presence of the glutamate-receptor antagonists DNQX and AP-5, TTX caused a 50% decrease in the frequency of IPSCs (i.e., mIPSC/sIPSC) in kainate-treated rats in both acute and chronic groups, which indicates persistent spike activity in interneurons of kainate-treated rats after pharmacological blockade of all glutamatergic input (i.e., these interneurons remained quite active, even without input from glutamatergic synapses). However, we also noticed that in the control groups, the TTX-induced reduction in IPSC frequency appeared different between the 4-7 day and the >3 month groups (~50% and ~25%, respectively), which may reflect a difference in the spontaneous activity among interneurons in the absence of excitatory input (i.e., some are more spontaneously active and some are less). Overall, the data presented here suggest that at least some interneurons generate action potential activity even when all glutamatergic input is eliminated pharmacologically (i.e., loss of excitatory input to interneurons does not make all of them “dormant”). In fact, the present data appear to support an opposite hypothesis, that the surviving interneurons after status epilepticus may fire more action potentials to compensate the lost input to dentate granule cells from other interneurons.

*Alteration of IPSC amplitude in rats 4-7 days but not >3 months after kainate treatment*

In general, the amplitude of mIPSCs depends on both quantal size (i.e., the amount of transmitter released from presynaptic terminals) and the number/density of postsynaptic receptors. At a central synapse, transmitter from a single vesicle is thought
to be sufficient to saturate postsynaptic receptors, and thus the number of available
postsynaptic receptors is the major determinant of mIPSC amplitude (Edwards et al.,
1990; Nusser et al., 1997). Several studies have suggested that inhibitory responses are
smaller in amplitude immediately after experimental status epilepticus (e.g., Sloviter,
1991; Hellier et al., 1999). Others have reported increased IPSC amplitude in dentate
granule cells from kindled (Nusser et al., 1998) or pilocarpine-treated animals (Brooks-
Kayal et al., 1998; Kobayashi and Buckmaster, 2003), possibly due to an upregulation of
postsynaptic GABA<sub>A</sub>-receptors (Nusser et al., 1998) or an alteration of the postsynaptic
GABA<sub>A</sub>-receptor subunits (Brooks-Kayal et al., 1998). In the present study, sIPSC and
mIPSC amplitude were observed to have increased 4-7 days, but not >3 months, after
kainate treatment. In particular, mIPSC amplitude was significantly increased and there
was a trend to larger amplitude for the sIPSCs. The change in the amplitude of IPSCs (at
least mIPSCs) suggests a transient up-regulation or alteration of the postsynaptic GABA
receptors shortly after status epilepticus, which may also be a compensatory response to a
reduction of inhibitory synaptic input to the dentate granule cells.

*Changes in IPSC kinetics*

The mean 10-90% rise time of IPSCs was not significantly altered at either 4-7
days or >3 months after kainate treatment compared to controls. These data appear to
suggest that the dentate granule cells have lost proportionally both peri-somatic and distal
inhibitory input. The prolonged decay time constant after kainate treatment suggest an
alteration in postsynaptic GABA receptors, which may also be a compensatory response
to the lost inhibitory input. The charge transfer of IPSCs (i.e., $pA \times ms$) is determined by both amplitude ($pA$) and decay time (ms). The increased charge transfer of IPSCs in the present study is consistent or in parallel with the observed increase of the IPSC amplitude (compare Fig. 6 and Fig. 8B), suggesting the increase in charge transfer was largely due to an increase in IPSC amplitude. The contribution of the prolonged decay time to the increase of charge transfer may be minor, as the same degree of prolongation failed to cause a significant increase in charge transfer in rats >3 months after kainate treatment (Fig. 8) where the IPSC amplitude was not increased (Figs. 6). However, both the increase of IPSC amplitude and prolongation of the decay time constant may serve to compensate for a reduction in the number of inhibitory input after kainate-induce status epilepticus.

Relevance to epileptogenesis

In the present study, a moderate reduction of inhibitory synaptic input to dentate granule cells (i.e., decreased mIPSC frequency) was observed shortly after kainate-induced status epilepticus, and this reduction of functional interneuron input appeared to be retained during the course of epileptogenesis. How important is this reduction of inhibitory synaptic input to epileptogenesis? One may postulate that this moderate loss of inhibitory input does not normally lead to the overt generation of seizure activity, since spontaneous seizures are rare immediately after kainate-induced status epilepticus and they increase in frequency in a progressive manner for several months in this model (Hellier et al., 1998, 1999). Moreover, compensatory mechanisms from both the
presynaptic (e.g., increase of interneuron firing) and postsynaptic side (e.g., GABA<sub>A</sub> receptor changes lead to an increased amplitude and prolongation of the decay time) may offset the reduction of inhibitory input. This suggests that other mechanisms are involved in epileptogenesis, such as sprouting and new recurrent excitation (Lynch and Sutula, 2000; Molnar and Nadler, 1999; Wuarin and Dudek, 2001; Scharfman et al., 2003), which would contribute to the enhanced propensity for epileptiform activity (Patrylo et al., 1999; Tauck and Nadler, 1985; Wuarin Dudek, 1996, 2001). Increased recurrent excitation in cortical networks can be controlled or masked by strong inhibition (Christian and Dudek, 1988; Miles and Wong, 1987); loss of inhibitory input may lead to inadequate control of the reorganized networks of excitatory granule and pyramidal cells, and thus facilitate synchronized seizure activity. Moreover, for the dentate gyrus, even when the sIPSC frequency is not greatly reduced (as observed in this study), the postsynaptic GABA<sub>A</sub> receptors in granule cells may become more sensitive to Zn<sup>2+</sup> during epileptogenesis (Cohen et al, 2003; Gibbs et al, 1997), which may make the inhibition more susceptible to collapse due to abundant Zn<sup>2+</sup> release at the sprouted mossy fiber terminals (but see Dudek, 2001; Molnar and Nadler, 2001). The data presented here support the hypothesis that kainate-induced status epilepticus causes a reduction of the inhibitory input to dentate granule cells, but they also suggest that the alterations of the inhibitory system during epileptogenesis may be quite complex and may potentially involve compensatory mechanisms. These studies highlight the future need to employ experimental analyses that focus on interneurons and the inhibitory network.
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FIGURE LEGENDS

Fig. 1. Examples of sIPSCs and mIPSCs recorded in dentate granule cells from rats 4-7 days after saline (A) or kainate treatment (B). The top trace in each panel is a 12 min (A) or a 14 min (B) recording of IPSCs. The boxed parts of the recordings are shown in expanded scale below. 1 and 2: sIPSCs; 3 and 4: mIPSCs; 5: IPSCs disappeared in bicuculline. Note robust sIPSCs and mIPSCs were present in both saline- and kainate-treated rats 4-7 days after treatment.

Fig. 2. Quantitative analyses of sIPSCs and mIPSCs in saline- and kainate-treated rats 4-7 days after treatment. A1: histograms of inter-sIPSC intervals from the saline (upper) or kainate (lower) groups. Values were logarithmically transformed. Both histograms fit a Gaussian distribution. The histograms of inter-sIPSC interval for the two groups were nearly identical and the cumulative distributions revealed no difference (A2, upper plot, also shown in expanded scale in lower, p>0.05, KS-test). B1: in contrast, the peak of the histogram in the kainate group (lower) was clearly shifted toward longer mIPSC intervals, compared with the saline group (dotted line). Similarly, the cumulative distributions of inter-mIPSC intervals of the saline and kainate groups were significantly different (B2, upper plot, also shown in expanded scale in lower trace, p<0.05, KS-test).

Fig. 3. Representative sIPSCs and mIPSCs recorded in dentate granule cells from rats >3 months after saline (A) or kainate treatment (B). Like in Fig. 1, the top traces in A and B is a 18 min or a 15 min recording of IPSCs, respectively. Lower traces (1-5) are
expansion of the boxed parts of recordings in the top traces, which show sIPSCs (1 and 2), mIPSCs (3 and 4) and the disappearance of these currents in bicuculline (5).

**Fig. 4.** Comparison of the inter-IPSC interval distributions in saline- and kainate-treated rats >3 months after treatment. *A1* and *A2*: the histograms of inter-sIPSC intervals for the two groups were very close (*A1*), and the cumulative distributions of the two groups were identical (*A2*, upper and lower plots, shown at different scales, p>0.05, KS-test). Similar to the rats 4-7 days after treatment, the peak of the histogram of inter-mIPSC interval in the kainate group (*B1*, lower) was clearly shifted to the right (i.e., longer interval), compared with the saline controls (dotted line). The cumulative distributions of inter-mIPSC intervals from the saline and the kainate group were significantly different (*B2*, upper and lower plots shown at different scales, p<0.05, KS-test).

**Fig. 5.** Summary of the mean mIPSC and sIPSC frequency in rats 4-7 days versus >3 months after treatment. *A*: the mean sIPSC frequency averaged across cells in rats 4-7 days after treatment were similar for the kainate and saline groups, whereas the mean mIPSC frequency of the kainate-treated rats was significantly lower than in mIPSC frequency for the saline controls. *B*: rats >3 months after saline or kainate treatment exhibited a similar pattern of IPSC frequency change (i.e., the mean sIPSC frequency was unaltered, but the mean mIPSC frequency was reduced). Asterisk indicates p<0.05, Student’s t-test. Note the frequency between the four sIPSCs groups was not significantly different (p>0.05, ANOVA), and the mIPSC frequency of the two control groups in *A* and *B* was similar (p>0.05, t-test).
Fig. 6. Summary of the mean IPSC amplitude in rats 4-7 days versus >3 months after treatment. A: unlike the mIPSC frequency, both the mean sIPSC and mIPSC amplitude in rats 4-7 days after kainate treatment tended to increase, but only the mean mIPSC amplitude exhibited a significant increase. B: in contrast, both sIPSC and mIPSC amplitudes in rats >3 months after kainate treatment were similar to that of saline treated controls. Mean amplitude was averaged across cells in each group. Asterisk indicates p<0.05, Student’s t-test.

Fig. 7. Analysis of IPSC kinetics: rise time. A: the mean 10-90% rise time of both sIPSCs and mIPSCs in rat 4-7 days after kainate treatment was close to their controls (p>0.05, t-test). B: similarly, the 10-90% rise time of sIPSCs and mIPSCs in rats >3 months after kainate treatment was not significantly different from their controls (p>0.05, t-test).

Fig. 8. Analysis of IPSC kinetics: decay time constant and charge transfer. A: in rats at both 4-7 days and >3 months after kainate treatment, the mean decay time constant of mIPSCs was slightly but significantly longer than their controls. B: the mean charge transfer of mIPSCs (represented by the area of the currents) in rats 4-7 days after kainate treatment was significantly increased. In contrast, mIPSCs in rats >3 months after kainate treatment showed a trend towards an increase in charge transfer that was not significantly different from controls. * p<0.05, ** p<0.01, Student’s t-test.
Figure 1.

A

1 2 TTX (2 µM) 3 4 5 Bic (30 µM)

1 2 3 4 5 sIPSCs

Saline (4-7 days)

3 mIPSCs

4

5

100 pA

2 min

B

1 2 TTX (2 µM) 3 4 5 Bic (30 µM)

1 2 3 4 5 sIPSCs

Kainate (4-7 days)

3 mIPSCs

4

5 100 pA

1 s
Figure 2.

**A1**

Number of Events

sIPSCs

Saline (4-7 days)

Kainate (4-7 days)

**B1**

Number of Events

mIPSCs

Saline (4-7 days)

Kainate (4-7 days)

Log$_{10}$ [interval] (ms)
Figure 3.

A

1. Saline (>3 months)
2. TTX (2 µM) Bic (30 µM)
3. sIPSCs
4. mlIPSCs
5. 200 pA

B

1. Kainate (>3 months)
2. TTX (2 µM) Bic (30 µM)
3. sIPSCs
4. mlIPSCs
5. 200 pA
Figure 4.

(A1) Number of events for sIPSCs.

(B1) Number of events for mIPSCs.

(A2) Cumulative fraction for sIPSCs.

(B2) Cumulative fraction for mIPSCs.
Figure 5.

Graph A:
- **Control (n = 17)**
- **Kainate (n = 19)**

Graph B:
- **Control (n = 19)**
- **Epileptic (n = 18)**

4-7 days:
- sIPSC
- mIPSC

>3 months:
- sIPSCs
- mIPSCs

* indicates significant difference.
Figure 6.

A

![Graph showing comparison between control (n = 17) and Kainate (n = 19) conditions for IPSC amplitude in 4-7 days.]

B

![Graph showing comparison between control (n = 19) and Epileptic (n = 18) conditions for IPSC amplitude in >3 months.]

Note: The graphs illustrate a significant increase in IPSC amplitude for Kainate and Epileptic conditions compared to controls.
Figure 7.

A

\[\text{10-90\% Rise Time (ms)}\]

- Control (n=17)
- Kainate (n=19)

B

\[\text{10-90\% Rise Time (ms)}\]

- Control (n=19)
- Epileptic (n=18)

4-7 days

>3 months
Figure 8.

A

mIPSCs

Decay Time Constant (ms)

Control
Kainate

<table>
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<th></th>
<th>4-7 days</th>
<th>&gt;3 months</th>
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<tbody>
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<td>Control</td>
<td>n=17</td>
<td>n=19</td>
</tr>
<tr>
<td>Kainate</td>
<td>n=19</td>
<td>n=18</td>
</tr>
</tbody>
</table>

B

mIPSCs

Charge Transfer (pA*ms)

Control
Kainate

<table>
<thead>
<tr>
<th></th>
<th>4-7 days</th>
<th>&gt;3 months</th>
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<tbody>
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