Mechanisms Underlying the Enhancement of Excitatory Synaptic Transmission in Basolateral Amygdala Neurons of the Kindling Rat

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Shoji, Y., E. Tanaka, S. Yamamoto, H. Maeda, and H. Higashi. Mechanisms underlying the enhancement of excitatory synaptic transmission in basolateral amygdala neurons of the kindling rat. J. Neurophysiol. 80: 638–646, 1998. To elucidate the mechanism underlying epileptiform discharges in kindled rats, synaptic responses in kindled basolateral amygdala neurons in vitro were compared with those from control rats by using intracellular and whole cell patch-clamp recordings. In kindled neurons, electrical stimulation of the stria terminalis induced epileptiform discharges. The resting potential, apparent input resistance, current-voltage relationship of the membrane, and the threshold, amplitude, and duration of action potentials in kindled neurons were not different from those in control neurons. The electrical stimulation of stria terminalis elicited excitatory postsynaptic potentials (EPSPs) and DL-2-amino-5-phosphono-pentanoic acid (AP5)-sensitive and 6-cyano-7-nitroquinolinoxaline-2,3-dione (CNQX)-sensitive excitatory postsynaptic currents (EPSCs). The amplitude of evoked EPSPs and of evoked AP5-sensitive and CNQX-sensitive EPSCs were enhanced markedly, whereas fast and slow inhibitory postsynaptic potentials (IPSPs) induced by electrical stimulation of lateral amygdaloid nucleus were not significantly different. The rise time and the decay time constant of the evoked CNQX-sensitive EPSCs were shortened, whereas the rise time of the evoked AP5-sensitive EPSCs was shortened, but the decay time constants were not significantly different. In both tetrodotoxin (TTX)-containing medium and low Ca2+ and TTX-containing medium, the frequency and amplitude of spontaneous EPSCs were increased in kindled neurons. These increases are presumably due to nearly synchronous multiquantal events resulted from the increased probability of Glu release at the nerve terminals. The rise time of evoked CNQX- and AP5-sensitive EPSCs and the decay time constant of evoked CNQX-sensitive EPSCs were shortened, suggesting that excitatory synapses at the proximal dendrite and/or the soma in kindled neurons may contribute more effectively to generate evoked EPSCs than those at distal dendrites. In conclusion, the increases in the amplitudes of spontaneous and evoked EPSCs and in the frequency of spontaneous EPSCs may contribute to the epileptiform discharges in kindled neurons.

INTRODUCTION

Kindling is currently a popular and useful animal model of epilepsy. Electrical kindling in the limbic system in rodents generates complex-partial seizures (Bradford 1995; Lothman et al. 1991). Brief electrical stimulation of the amygdala or hippocampus once per day for more than a week produces kindling and, if maximally kindled (stage 5 of Racine 1972), epileptogenesis continues for long periods of time. The development of kindling can be prevented or reduced by injection of N-methyl-D-aspartate (NMDA) receptor antagonists (Croucher et al. 1988; Durmuller et al. 1994; Sato et al. 1988) or non-NMDA receptor antagonists (Croucher et al. 1988; Durmuller et al. 1994) respectively, 5–30 min before application of the kindling stimulus, suggesting that both non-NMDA and NMDA receptors play a key role in the electrical kindling process.

In the hippocampus, the initial electrographic seizures evoked by kindling induce a long-lasting increase in excitatory synaptic transmission (Sutula and Steward 1986), which is at least partly mediated by the NMDA receptor channels in granule cells of the dentate gyrus (Mody and Heinemann 1987; Mody et al. 1988; Sutula et al. 1996). This initial physiological alteration is accompanied by a complex sequence of gene expression, which includes transient increases in expression of transcription factors (Dragunow and Robertson 1987; Hope et al. 1994; Hosford et al. 1995; Labiner et al. 1993; Morgan and Curran 1991; Shin et al. 1990) and slowly evolving changes in neurotrophins (Ernfors et al. 1991; Gall 1993; Gall and Isackson 1989; Khrestchatisky et al. 1995; Lindvall et al. 1994; Rashid et al. 1995), neurotrophic factor receptors (Bengzon et al. 1993; Bugra et al. 1994), and axonal growth-associated proteins (Bendotti et al. 1993; Meberg et al. 1993), as described previously (Sutula et al. 1996). These changes are followed by slow cellular alterations that include sprouting of the mossy fiber pathway in the dentate gyrus (Cavazos et al. 1992; Golarai and Sutula 1996; Represa and Ben-Ari 1992; Sutula et al. 1988, 1996).

The amygdala is one of the brain areas most sensitive to kindling-induced neuroplasticity (Lösch et al. 1995). Previous studies have demonstrated that for several weeks after the last amygdala-kindled seizure in vivo, spontaneous and evoked epileptiform bursting can be recorded in vivo (Goddard et al. 1969; Kawakami et al. 1990; Matsuura et al. 1993; Racine 1972) and in vitro (Asproditi et al. 1992a,b; Gean et al. 1989; Holmes et al. 1996; Rainnie et al. 1992). In basolateral amygdala (BLA) neurons, kindling reduces the γ-amino butyric acid (GABA)-receptor–mediated inhibitory transmission (Asproditi et al. 1992b; Gean et al. 1989; Rainnie et al. 1992) and enhances both NMDA- and non-NMDA-receptor–mediated glutamatergic transmission (Gean et al. 1989; Rainnie et al. 1992). Nevertheless, it is still unclear whether the enhancement of the evoked fast excitatory postsynaptic potential (EPSP) in kindled neurons is due to loss of inhibitory input from GABAergic neurons and/or an increase in glutamate (Glu) release from excitatory nerve terminals or whether changes in the number and characteristics of the Glu receptors are involved in the enhanced fast EPSP in kindled neurons.

To elucidate the mechanism underlying epileptiform burst discharges in kindled rats, the present study has examined...
whether resting and active membrane properties are altered, whether synaptic release of Glu is increased, and whether GABA-mediated inhibitory postsynaptic potentials (IPSPs) are depressed in BLA kindled neurons. Preliminary accounts of some data have been presented previously (Shoji et al. 1995).

**METHODS**

**Kindling**

Adult male Wistar rats (150–200 g) were stereotaxically implanted with an insulated bipolar electrode for stimulation and recording under anesthesia by intraperitoneal administration of pentobarbital sodium at a dose of 50 mg/kg body wt. The electrode was implanted in the right amygdala (2.8-mm posterior to bregma, 5.0-mm lateral to the midline, and 8.0-mm ventral to the skull) (Paxinos and Watson 1982). After a postoperative recovery period of ≥7 days, animals (n = 40) were stimulated once daily (a 1-s train of 1-msec biphasic rectangular pulses delivered at a frequency of 60 Hz) at an intensity of the initial threshold for afterdischarge. Stimulation was applied until the animals had produced at least five consecutive stage 5 seizures in which rearing and/or falling were seen (Racine 1972). Controls consisted of animals that were implanted but never stimulated (n = 30) and unimplanted age-matched animals (n = 26).

**Slice preparation**

Kindled rats, sham-operated or unoperated rats (350–450 g) were killed with a heavy blow to the chest under diethylether anesthesia. Kindling rats were used 4 ± 8 wk after the last kindled and the electrode was kept within 100 μm of the tissue surface. The brain subsequently was hemisected and transversely dissected lateral olfactory tract, and paraventricular nucleus and interanteromedial nucleus of the thalams (Ottersen et al. 1986).

All drugs were applied by superfusion in the ACSF and introduced into the recording chamber by means of a three-way stopcock. When switching the superfusing media, there was a delay of 15–20 s before the new medium reached the chamber due to the volume of the connecting tubing. Thus the chamber was filled with the test solution ~30 s after switching the three-way cock. The following drugs were used in this study: ibotenic acid, 5,7-dihydroxytryptamine (5,7-DHT), bicuculline methiodide, saclofen, quinoxaline-2,3-dione (CNQX) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; both from Tocris Bioscience). NMDA, bicuculline methiodide, saclofen, and muscimol (all from Sigma); tetrodotoxin (TTX, from Wako); pentobarbital (from Aventis); and 6-cyano-7-nitroquinoxaline-2,3-dione (CNOX) and 5,7-DHT were added to the internal solution for improving space and reducing currents (EPSCs). K gluconate or KCl was substituted by equimolar amounts of CsSO₄ or CsCl, respectively, for improving space clamp, and guanosine 5′-O-(2-thiodiphosphate) (GDPβS; 1 mM) was added to the internal solution for preventing the generation of postsynaptic responses that are mediated via activation of G protein. The pH and osmolality of the solution were adjusted to 7.1 and 285 mosm, respectively. The resistance of patch electrodes was 4–8 MΩ. Junction potentials were compensated after placing the pipette in the bath. Cells were voltage clamped at ~80 mV. In some experiments, membrane potentials were held at ~60 mV because holding currents were minimal (0 to ~10 pA). At this voltage level, EPSCs were observed as inward current and inhibitory postsynaptic currents (IPSCs) were observed as outward current. Membrane currents were amplified (1–10 mV/pA), filtered at 2 kHz and digitized at 20 kHz. The membrane current and voltage were monitored continuously on a digital oscilloscope.

Electrical stimuli were applied using single bipolar tungsten steel electrodes of which diameter was 100 μm, and the distance between the two electrodes was 200 μm. The electrode was placed onto the ST (1.5 ± 0.1 mm from the recording electrode) or the lateral amygdaloid nucleus (LA; 0.3 ± 0.1 mm from the recording electrode) and the electrode was kept within 100 μm of the tissue surface. The ST pathway consists of afferent fibers from the piriform cortex, the lateral entorhinal area, the subiculum, nucleus of the lateral olfactory tract, and paraventricular nucleus and interanteromedial nucleus of the thalams (Ottersen et al. 1986).

Whole cell patch-clamp recordings also was performed with a patch-clamp amplifier (Axon Instruments, Axopatch 200A). The patch pipette was made of a thin-walled fiber-filled glass (1.5 mm OD). For whole cell patch-clamp recordings, the electrode was filled with an artificial internal solution of the following composition (in mM): 135 K gluconate, 5 KCl, 0.5 CaCl₂, 2 MgCl₂, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 5 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, and 5 ATP. In experiments to record evoked excitatory postsynaptic currents (EPSCs), K gluconate or KCl was substituted by equimolar amounts of CsSO₄ or CsCl, respectively, for improving space clamp, and guanosine 5′-O-(2-thiodiphosphate) (GDPβS; 1 mM) was added to the internal solution for preventing the generation of postsynaptic responses that are mediated via activation of G protein. The pH and osmolality of the solution were adjusted to 7.1 and 285 mosm, respectively. The resistance of patch electrodes was 4–8 MΩ. Junction potentials were compensated after placing the pipette in the bath. Cells were voltage clamped at ~80 mV. In some experiments, membrane potentials were held at ~60 mV because holding currents were minimal (0 to ~10 pA). At this voltage level, EPSCs were observed as inward current and inhibitory postsynaptic currents (IPSCs) were observed as outward current. Membrane currents were amplified (1–10 mV/pA), filtered at 2 kHz and digitized at 20 kHz. The membrane current and voltage were monitored continuously on a digital oscilloscope.

Intracellular and whole cell patch-clamp recording techniques

A slice was placed on a nylon net in a recording chamber and immobilized with a titanium grid placed on its upper surface. The slice was fully submerged and maintained at 32 ± 1°C (mean ± SD) with continuously superfused ACSF (7–8 ml/min).

Intracellular recordings were made from neurons in BLA nucleus. Intracellular recording electrodes (60–100 MΩ) were filled with potassium acetate (2 M) in most experiments for recording evoked postsynaptic potentials (PSPs) and KCl (3 M) in some experiments for recording membrane properties. Signals were amplified with a high-input impedance bridge amplifier (CEZ-3100, Nihon Kohden). The membrane potential and current were monitored with a digital oscilloscope (VC-11, Nihon Kohden) and recorded on a DC chart recorder (WS-641 G, Nihon Kohden). Intracellular recordings were considered acceptable if neurons exhibited overshooting action potentials and showed stable membrane potentials more negative than ~60 mV in the absence of a DC holding current. The bridge balance was monitored carefully throughout the experiments and adjusted when necessary.

Whole cell patch-clamp recording also was performed with a patch-clamp amplifier (Axon Instruments, Axopatch 200A). The patch pipette was made of a thin-walled fiber-filled glass (1.5 mm OD). For whole cell patch-clamp recordings, the electrode was filled with an artificial internal solution of the following composition (in mM): 135 K gluconate, 5 KCl, 0.5 CaCl₂, 2 MgCl₂, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 5 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, and 5 ATP. In experiments to record evoked excitatory postsynaptic currents (EPSCs), K gluconate or KCl was substituted by equimolar amounts of CsSO₄ or CsCl, respectively, for improving space clamp, and guanosine 5′-O-(2-thiodiphosphate) (GDPβS; 1 mM) was added to the internal solution for preventing the generation of postsynaptic responses that are mediated via activation of G protein. The pH and osmolality of the solution were adjusted to 7.1 and 285 mosm, respectively. The resistance of patch electrodes was 4–8 MΩ. Junction potentials were compensated after placing the pipette in the bath. Cells were voltage clamped at ~80 mV. In some experiments, membrane potentials were held at ~60 mV because holding currents were minimal (0 to ~10 pA). At this voltage level, EPSCs were observed as inward current and inhibitory postsynaptic currents (IPSCs) were observed as outward current. Membrane currents were amplified (1–10 mV/pA), filtered at 2 kHz and digitized at 20 kHz. The membrane current and voltage were monitored continuously on a digital oscilloscope.

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**Data analysis**

The apparent input resistance was monitored by passing small hyperpolarizing pulses (0.1–0.2 nA, 250 ms) through the recording electrode. The amplitude of the action potential was measured from the resting membrane potential to the peak potential. The duration of the action potential was measured at the threshold level.

Spontaneous EPSPs were analyzed off-line using the AxoGraph data analysis program (Axon Instruments). Events were semi-automatically detected in epochs of 20–60 s in duration using an adjustable amplitude threshold, typically set at ~4 pA, and held constant for a given experiment. Synaptic events were selected in a blinded
Membrane properties of control and kindled neurons in basolateral amygdaloid nucleus

Twenty-two kindled rats and 12 sham-operated and 26 unoperated rats were used in the following experiments. Resting and active membrane properties were compared between control and kindled BLA neurons recorded by conventional intracellular microelectrodes filled with potassium acetate (2 M) or KCl (3 M). The resting membrane potential and the apparent input resistance in kindled neurons were not different from those in control neurons. The resting membrane potential and the apparent input resistance in kindled neurons were $-69 \pm 4$ (SD) mV ($n = 57$) and $52 \pm 15$ MΩ ($n = 57$) and in control neurons were $-69 \pm 4$ mV ($n = 113$) and $49 \pm 14$ MΩ ($n = 113$), respectively. The threshold, the amplitude, and the duration of the action potential in kindled neurons were also not different from those in control neurons. The threshold, the amplitude, and the duration of the action potential in kindled neurons were $-57 \pm 1$ mV, $84 \pm 3$ mV, and $0.9 \pm 0.1$ ms ($n = 15$) and those in control neurons were $-57 \pm 1$ mV, $83 \pm 4$ mV, and $1.0 \pm 0.2$ ms ($n = 15$), respectively. The action potential was reversibly abolished by TTX (0.3 M, $n = 8$). In control and kindled BLA neurons, the distributions of the apparent input resistances were not different from a normal distribution ($\chi^2$ square test was used to test the distribution pattern. $\chi^2 = 12.811$, df = 7, and $P = 0.0768$ for control and $\chi^2 = 7.934$, df = 7, and $P = 0.3385$ for kindled neurons), suggesting that neurons recorded from kindled basolateral amygdaloid slices had the same passive properties as control cells.

There was no significant difference in the zero current membrane potential between control ($-63 \pm 3$ mV, $n = 48$) and kindled neurons ($-54 \pm 5$ mV, $n = 50$) recorded with the whole cell patch-clamp method. In some neurons, I-V relations were obtained by passing slow ramp command pulses (1–2 mV/s) through the recording electrodes. The I-V curves at the membrane potential between $-50$ and $-100$ mV were linear, and the conductances measured at the potential range in control ($7 \pm 3$ nS; $n = 7$) and kindled ($6 \pm 2$ nS; $n = 9$) neurons were not significantly different.

Evoked EPSPs and evoked IPSPs

PSPs were compared between control and kindled BLA neurons recorded by conventional intracellular microelec-

trades filled with potassium acetate (2 M). PSPs were elicited by brief electrical stimulation (100 μs in duration) of the ST 1.5-mm away from the recorded control and kindled neurons. In control neurons, multiphasic PSPs consisting of fast EPSPs, a fast IPSP, and a subsequent slow IPSP were recorded at resting membrane potentials of $-70$ mV. As reported previously, the fast EPSPs are due to activation of both NMDA- and non-NMDA-type Glu receptors, and the fast and slow IPSPs are mediated by activation of GABA$_A$ and GABA$_B$ receptors, respectively (Rainnie et al. 1991a,b).

On the other hand, stimulation in the ST at the intensity that elicited PSPs in control neuron, or even at lower intensities, elicited epileptiform discharges in the majority of kindled neurons. Thus the stimulus intensity of 6 V elicited epileptiform discharges in 6 of 15 kindled neurons tested, long-lasting EPSPs with a single spike in 2 neurons, and subthreshold EPSPs in the remaining 7 neurons. Moreover, the amplitude ($17 \pm 2$ mV, $n = 7$, $P < 0.001$) of the subthreshold EPSP recorded at $-70$ mV in the kindled neurons was significantly greater than that ($9 \pm 4$ mV, $n = 9$) elicited by the same intensity of 6 V in control neurons. The minimal stimulus intensities required to elicit synaptic responses in kindled neurons ($4 \pm 2$ V, $n = 15$) were significantly lower than those in control neurons ($9 \pm 3$ V, $n = 27$, $P < 0.01$).

To examine the differences in the directly elicited IPSPs between control and kindled neurons, a fast IPSP and a subsequent slow IPSP elicited by single stimulation of the lateral amygdala (LA) nucleus were recorded in the presence of AP5 (50–100 μM) and CNQX (10–20 μM). Neither the directly elicited fast IPSP nor the slow IPSP was different between kindled neurons and control neurons. The amplitudes of the fast IPSP and slow IPSP in kindled neurons were $9 \pm 1$ mV and $7 \pm 1$ mV ($n = 8$), respectively, and those in control neurons were $9 \pm 1$ mV and $7 \pm 3$ mV ($n = 18$), respectively.

Evoked EPSCs mediated by NMDA and non-NMDA receptors

Two kindled rats and 16 sham-operated rats were used in the following experiments. To investigate the contribution of NMDA and non-NMDA receptors to the augmentation of fast EPSPs, AP5- and CNQX-sensitive EPSCs were recorded by whole cell voltage-clamp method in the presence of combination of bicuculline (10–20 μM), sullofen (100 μM), and either CNQX (10–20 μM) or AP5 (50–100 μM). The CNQX-sensitive EPSC was increased in amplitude and duration when the membrane was hyperpolarized and abolished by CNQX (10–20 μM). On the other hand, the AP5-sensitive EPSC was decreased in amplitude and duration when the membrane was hyperpolarized in the presence of Mg$^{2+}$ (1.2 mM) and abolished in the presence of AP5 (50–100 μM). Figure 1 illustrates superimposed traces of typical CNQX-sensitive EPSCs (Fig. 1A) and AP5-sensitive EPSCs, (Fig. 1C) evoked by graded intensities (3–15 V) of single focal stimuli in control and kindled neurons. When the stimulus was progressively increased (>3 V), smoothly graded both CNQX- and AP5-sensitive EPSCs were evoked. These EPSCs showed monosynaptic characteristics; i.e., they were constant in latency and shape, and there were no failures during a train of 20 stimuli at 20 Hz. At intensities >12
V, however, both types of EPSCs often were elongated or polysynaptic; i.e., compound EPSCs were evoked. Figure 1, B and D, summarizes these experiments. In kindled neurons, CNQX- and AP5-sensitive EPSCs were elicited by low-intensity (3 V) focal stimuli, whereas both types of EPSCs were evoked by high intensities (6 V) in control neurons. In kindled neurons, the mean amplitudes of both CNQX- and AP5-sensitive EPSCs were significantly greater than in control neurons at each stimulus intensity between 6 and 18 V (Fig. 1, B and D; \( P < 0.05 \)). At an intensity of 18 V, the amplitudes of CNQX- and AP5-sensitive EPSCs in kindled neurons were increased to 572 \( \pm \) 100% (\( n = 6 \)) and 378 \( \pm \) 178% (\( n = 6 \)), respectively, of each control.

Because AP5- and CNQX-sensitive EPSCs elicited by intensities \(< 12 \) V were not contaminated by polysynaptic events, the rise time and the decay time constant of both types of EPSCs in kindled neurons were compared with those of EPSCs in control neurons; the rise time and decay time constant for both CNQX- and AP5-sensitive EPSCs were measured at similar amplitude (from 8 to 9 pA) of both EPSCs in kindled and control neurons (Table 1). The rise times for both CNQX- and AP5-sensitive EPSCs in kindled neurons were much faster than those in control neurons. The decay time constant of CNQX-sensitive EPSCs was also faster in kindled neurons, but that of AP5-sensitive EPSCs was not different between control and kindled neurons.

**Spontaneous synaptic activity**

Six kindled rats and six sham-operated rats were used in the following experiments. To elucidate whether an increase in the amplitude of CNQX-sensitive EPSCs in kindled neurons is caused by presynaptic and/or postsynaptic mechanisms, spontaneous EPSCs in control neurons were compared with those in kindled neurons in the presence of TTX (1 \( \mu M \)) under whole cell voltage-clamp. Spontaneous EPSCs were recorded at a holding potential of \(-60 \) mV, at which potential EPSCs were observed as inward current and IPSCs were observed as outward current. Spontaneous EPSCs were recorded in all neurons tested and were abolished by CNQX (5 \( \mu M, n = 12 \)), and only baseline noise remained in control (Fig. 2) and kindled rats (not shown). On the other hand, spontaneous IPSCs were abolished by bicuculline (10 \( \mu M \); not shown). The amplitude of baseline noise was distributed from \(-4 \) to \(-4 \) pA with a normal distribution, and the mode, the mean amplitude, and the variance were 0 pA, \(-0.08 \) pA, and 2.47 pA\(^2\), respectively. The frequency of spontaneous IPSCs (<1 Hz) was always lower than that of spontaneous EPSCs (>5 Hz) in control neurons.

**TABLE 1. Rates of rise and decay time constants of evoked EPSCs in control and kindled basolateral amygdala neurons**

<table>
<thead>
<tr>
<th>Evoked EPSCs at Threshold Stimuli</th>
<th>Rise Time, ms</th>
<th>Decay Time Constant, ms</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNQX sensitive</td>
<td>5.2 ( \pm ) 2.0 (28)</td>
<td>16.7 ( \pm ) 5.6 (28)</td>
</tr>
<tr>
<td>AP5 sensitive</td>
<td>7.5 ( \pm ) 3.6 (26)</td>
<td>32.1 ( \pm ) 7.2 (26)</td>
</tr>
<tr>
<td>Kindled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNQX sensitive</td>
<td>4.1 ( \pm ) 1.7 (23)</td>
<td>13.6 ( \pm ) 4.1 (23)</td>
</tr>
<tr>
<td>AP5 sensitive</td>
<td>4.9 ( \pm ) 2.2 (40)</td>
<td>34.3 ( \pm ) 12.7 (40)</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SD with the number of neurons given in parentheses. Evoked excitatory postsynaptic currents (EPSCs) were recorded by using the whole cell patch-clamp technique. Basolateral amygdala (BLA) neurons were held at a holding potential of \(-80 \) mV. Rise times were measured from 10 to 90% of the peak amplitude of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)- and DL-2-amino-5-phosphonopentanoic acid (AP5)-sensitive EPSCs at the rising phase. The decay phases, from 90 to 10% of the peak amplitude of CNQX- and AP5-sensitive EPSCs, were well fitted by single exponential curves, and decay time constants of both types of EPSCs were calculated. The threshold stimulus for the control and the kindling neurons was the intensity of the focal stimulus at stria terminalis, which was 6 V for control and 3 V for kindling neurons. \( * P < 0.05 \) and \( ** P < 0.01 \), nonparametric Kolmogorov-Smirnov test.
The distribution of amplitudes was skewed in both control and kindled neurons (Fig. 3B), but in kindled neurons, the amplitude distributions were skewed toward larger amplitudes than in control neurons. Figure 3C shows the normalized cumulative amplitude distributions for control and kindled neurons. The cumulative distribution curve in kindled neurons was shifted to the larger amplitude in comparison with the control (Fig. 3C); maximum difference of the amplitude was 0.16 by Kolmogorov-Smirnov test. The mean amplitude in kindled neurons was also significantly greater than that in control neurons ($P < 0.05$; Table 2). Figure 3D shows that the normalized cumulative interevent interval distributions in kindled neurons was shifted to the lower interval in comparison with the control; the maximum absolute difference of the interevent interval was 0.20 by Kolmogorov-Smirnov test. The mean interevent interval in kindled neurons ($68 \pm 15$ ms, $n = 6$) was significantly shorter than that in control neurons ($112 \pm 32$ ms, $n = 6$, $P < 0.01$). On the other hand, the rise time and decay time constant of spontaneous EPSCs ($5.0 \pm 3.8$ and $9.5 \pm 5.4$ ms, respectively, $n = 480$ events) in kindled neurons were not significantly different from those ($5.4 \pm 3.5$ and $10.2 \pm 5.8$ ms, respectively, $n = 300$ events) in control neurons.

The increase in the mean amplitude of spontaneous EPSCs of kindled neurons in TTX-containing medium may be due to an increase in the quantal size and/or a shift of the amplitude distribution to the right, resulting from an increase in the Glu release probability (see DISCUSSION). Thus in the presence of TTX (1 $\mu$M), extracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]o) was reduced from 2.5 to 0.5 mM to examine spontaneous EPSCs in details. In control neurons, the frequency of spontaneous EPSCs ($9 \pm 3$ Hz, $n = 6$) in TTX-containing medium was reduced to $5 \pm 2$ Hz ($n = 6$) in TTX-containing low Ca$^{2+}$ medium. In kindled neurons, the frequency of spontaneous EPSCs ($15 \pm 3$ Hz, $n = 6$) in TTX-containing medium was reduced to $8 \pm 2$ Hz ($n = 6$) in TTX-containing low Ca$^{2+}$ medium. Mean amplitudes of spontaneous EPSCs in kindled and control neurons in TTX-containing medium and those in TTX-containing low Ca$^{2+}$ medium were summarized in Table 2. In control and kindled neurons, the mean amplitude of spontaneous EPSCs was significantly reduced in TTX-containing low Ca$^{2+}$ medium (by Kolmogorov-Smirnov test, $P < 0.05$). In a control neuron, the skew to large amplitudes was lost from the amplitude distribution in low Ca$^{2+}$ medium (Fig. 4A). In a kindled neuron, the skewness also was reduced in low Ca$^{2+}$ medium (Fig. 4B). However, in TTX-containing low Ca$^{2+}$ medium, the modes of the amplitude distribution of spontaneous EPSCs were not different from that of spontaneous EPSCs in TTX-containing medium. In 12 neurons tested, the mode was 5 ($n = 1$), 6 ($n = 4$), and 7 pA ($n = 1$) in control neurons and was 6 ($n = 3$) and 7 pA ($n = 3$) in kindled neurons.

In TTX-containing low Ca$^{2+}$ medium, the interevent interval of spontaneous EPSCs was significantly prolonged (Fig. 5A). Figure 5B shows the amplitude histogram of spontaneous EPSCs for control and kindled neurons. The distribution of the amplitude was skewed in both control and kindled neurons. The modes of the amplitude distribution was between 6 and 7 pA in control neurons and between 7 and 8 pA in kindled neurons. Figure 5C shows the normalized cumulative amplitude distributions for control and kindled neurons; maximum absolute difference of the amplitude was 0.15 by Kolmogorov-Smirnov test. The mean amplitude of spontaneous EPSCs in kindled neurons was significantly larger than that in control neurons (Table 2). Figure 5D shows the normalized cumulative interevent interval distributions of control and kindled neurons; the maximum absolute difference of the interevent interval was 0.15 by Kolmogorov-Smirnov test. The mean interevent interval ($125 \pm 33$ ms, $n = 6$) in kindled neurons was significantly shorter than that ($208 \pm 74$ ms, $n = 6$) in control neurons ($P < 0.01$).

**Discussion**

**Mechanisms for generating epileptiform discharge in kindled neurons**

The present study demonstrates that in kindled neurons, electrical stimulation of the stria terminalis induced epileptiform discharges. The resting potential, apparent input resistance, current-voltage relationship of the membrane, and the threshold, amplitude, and duration of action potentials in kindled neurons were not different from those in control
neurons. The amplitude of evoked monosynaptic EPSPs and of evoked AP5- and CNQX-sensitive EPSCs were enhanced markedly, whereas monosynaptic fast and slow IPSPs were not significantly different. These findings are consistent with the previous report that in kindled BLA neurons CNQX- and AP5-sensitive EPSPs are increased in amplitude (Rainnie et al. 1992) and are comparable with the previous finding that prolonged EPSP with burst firing is depressed in the presence of AP5 (Gean et al. 1989). In addition, the present study confirms the previous result that in kindled BLA neurons the fast IPSP evoked directly by the stimulus at the LA is not altered (Rainnie et al. 1992).

Ligand binding studies have demonstrated that AMPA or kainate binding sites (Okazaki et al. 1990), NMDA recognition sites, and the modulation of NMDA receptor channels by Mg$^{2+}$ (Jones and Johnson 1989) are not altered in rat kindled amygdala. Moreover, AMPA- or NMDA-induced inward currents are not increased in kindled BLA neurons (Shoji et al. 1995). It is likely, therefore, that the increase in amplitudes of CNQX- and AP5-sensitive EPSCs is probably due to increase in Glu release from the nerve terminals. Nevertheless, there are many controversial results in kindled hippocampal neurons; NMDA binding sites are decreased (Okazaki et al. 1989; Sircar et al. 1987), and the expression of NMDA splice isoforms is decreased in CA1, CA3, and lower blade of the dentate gyrus region (Kraus et al. 1994). In contrast, responses to NMDA are increased in CA3 neurons (Martin et al. 1992). The mean open time and the duration of burst and cluster of NMDA receptor channels are increased, and the affinity for Mg$^{2+}$ of NMDA receptor channels is lower (Köhrl et al. 1993). Moreover, NMDA-induced currents recorded with lack of ATP in the pipette solution under the whole cell patch-clamp are reduced in kindled neurons (Köhrl et al. 1993). Thus direct evidence for the augmentation of NMDA receptor activity is still lacking or inconclusive in kindled hippocampal neurons.

In dentate gyrus, muscimol-binding sites and flunitrazepam-binding sites are transiently increased within 24 h after the last electrical stimulation (Nobrega et al. 1989, 1990; Shin et al. 1985). In addition, the functional GABA$_A$ receptor channels are increased in the kindled rat dentate gyrus without any change in single-channel conductance or kinetics 24–48 h after the last seizure (Otis et al. 1994). However, the present study demonstrates that the monosynaptic fast and slow IPSPs were not significantly altered in kindled BLA neurons.

From all the results, it concluded that epileptiform burst discharge in kindled rats is resulted from augmentation of CNQX- and AP5-sensitive EPSPs, but not due to depression of GABA-mediated IPSPs.

**Mechanisms for augmentation of evoked and spontaneous EPSCs in kindled neurons**

The present study demonstrates that the rise time of evoked CNQX- and AP5-sensitive EPSCs and the decay

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**TABLE 2.** Mean amplitudes of spontaneous EPSCs in kindled and control neurons in TTX-containing and TTX-containing low Ca$^{2+}$ (0.5 mM) medium

<table>
<thead>
<tr>
<th>Condition</th>
<th>TTX (1 μM)/low Ca$^{2+}$ (0.5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean amplitude of spontaneous EPSCs, pA 8.7 ± 3.6 (6) 7.0 ± 1.8 (6)</td>
</tr>
<tr>
<td>Kindled</td>
<td>Mean amplitude of spontaneous EPSCs, pA 10.3 ± 6.3 (6)* 7.5 ± 1.8 (6)*</td>
</tr>
</tbody>
</table>

* Values are means ± SD with the number of neurons given in parentheses. Spontaneous EPSCs were recorded using the whole cell patch-clamp technique. BLA neurons were held at a holding potential of −60 mV. *P < 0.05, nonparametric Kolmogorov-Smirnov test. TTX, tetrodotoxin.
Recent studies provide evidence that perforant path kin- 
most likely that the Glu release probability in the excitatory 
and the effect of low Ca\(^{2+}\) neurons. Data are taken from 60 s of recording with 514 events for the suppressed and the major peak of distribution also was re-distribution in presence of TTX and 288 events for the distribution in duced in low Ca\(^{2+}\) solution with TTX. Its effect on amplitude histograms of is presumably due to nearly synchronous multiquantal events spontaneous EPSCs in control and kindled neurons. Holding potential 
(0.5 mM) on amplitude histograms of is presumably due to nearly synchronous multiquantal events 
spontaneous EPSCs in control and kindled neurons. Holding potential 
(0.5 mM) solution nerve terminals. This might follow if nonuniform release 
ar bouton/spine pair (see Edwards 1995). This is less likely 
separate active zones forming the synapse between a particu-
lar bouton/spine pair (see Edwards 1995). This is less likely 
cause in kindled rats, AMPA or kainate binding sites are 
not somatic synapses, in the medial amygdaloid nucleus 
are not altered in basolateral amygdala (Okazaki et al. 1990).

In addition, it is likely that the Glu release probability 
becomes more uniform after the new synaptic formation. 
Recent studies provide evidence that perforant path kin-
dling induces sprouting mossy fibers into the supragra-
nular molecular layer of the hippocampal dentate gyrus 
(Golarai and Sutula 1996; Sutula et al. 1996) and may increase recurrent excitation (Waurin and Dudek 1996).
Represa and Ben-Ari (1992) have reported that amygdalo-
doid kindling induces sprouting of mossy fibers and synap-
tic reorganization in the CA3 region of the hippocampus. 
Okada et al. (1993) have demonstrated that using the elec-
tron microscope, the number of dendritic synapses, but 
not somatic synapses, in the medial amygdaloid nucleus 
is reduced markedly in kindled rats.

In both TTX-containing medium and low Ca\(^{2+}\) and TTX-
containing medium the interevent intervals of spontaneous 
EPSCs were decreased in kindled neurons. The result that 
maximum absolute difference between control and kindled 
neurons in the interevent intervals of spontaneous EPSCs in 
TTX-containing medium was much greater than that in TTX-
containing low Ca\(^{2+}\) medium (cf. Fig. 3D with 5D) suggests 
that an increase in the release probability of Glu in kindled 
neurons. The increase in release probability of excitatory 
nerve terminals is probably due to an increased background 
Ca\(^{2+}\) concentration at the active zone in the terminals (see 
Zucker 1993; Zucker et al. 1991). Alternatively, the increase in frequency of spontaneous EPSCs may be due to multiple 
separate active zones forming the synapse between a particu-
lar bouton/spine pair (see Edwards 1995). This is less likely 
because in kindled rats, AMPA or kainate binding sites are 
not altered in basolateral amygdala (Okazaki et al. 1990).

In TTX-containing medium, the mean amplitude of sponta-
neous EPSCs were increased markedly in kindled neurons. The increase in the mean amplitude of spontaneous EPSCs 
is presumably due to nearly synchronous multiquantal events 
resulted from the increased probability of Glu release at the 
nerve terminals. This might follow if nonuniform release 
probability at separate release sites (Redman 1990; Redman 
and Walmsley 1983; Rosenmund et al. 1993) becomes more 
uniform after the formation of kindling. In fact, the skewed 
distribution in larger amplitudes of spontaneous EPSCs was 
suppressed and the major peak of distribution also was re-
duced in low Ca\(^{2+}\) and TTX-containing medium, especially 
in the kindled neurons (Fig. 4B). It is likely, therefore, that 
an increase in intracellular Ca\(^{2+}\) concentration at the active 
zone of the excitatory presynaptic terminals may induce uni-
form Glu release in kindled neurons.

There are three possibilities on the small increase in the 
mean amplitude of kindled neurons in low Ca\(^{2+}\) and TTX-
containing medium. One possibility is that glutamate content 
in the vesicle is slightly high in kindled neurons. This is, 
however, unlikely because in TTX-containing medium the 
mode of the amplitude distribution for each kindled neuron 
is not different from the each control neuron and only the 
number of neurons that shown high mode (7 pA) was in-
creased. Another possibility is that the affinity, number, and/or 
single channel conductance of AMPA/kainate type gluta-
mat e receptor/channels are increased. This is less likely be-
cause in kindled rats, AMPA or kainate binding sites are not 
alter ed in BLA (Okazaki et al. 1990) and responses to 
AMPA are not significantly altered between the control and 
kindled BLA neurons (Shoji et al. 1995). Therefore it is 
most likely that the Glu release probability in the excitatory

![Figure 4](http://jn.physiology.org/)

**Figure 4.** Effects of low Ca\(^{2+}\) (0.5 mM) on amplitude histograms of spontaneous EPSCs in control and kindled neurons. Holding potential was −60 mV. A: typical amplitude distribution histograms in the presence of TTX (1 μM; ■) and the effect of low Ca\(^{2+}\) (0.5 mM) solution with TTX (●) in the same control neuron. Data are taken from 60 s of recording with 337 events for the distribution in the presence of TTX and 266 events for the distribution in low Ca\(^{2+}\) solution with TTX. B: typical amplitude distribution histograms in the presence of TTX (●) and the effect of low Ca\(^{2+}\) solution with TTX (■) in the same kindled neuron. Data are taken from 60 s of recording with 514 events for the distribution in presence of TTX and 288 events for the distribution in low Ca\(^{2+}\) solution with TTX.
ENHANCEMENT OF EPSCs IN KINDLED AMYGDALA NEURONS

FIG. 5. Differences between control and kindled neurons in the amplitude and interevent interval of the spontaneous EPSCs recorded in low Ca\(^{2+}\) (0.5 mM) solution. Holding potential was −60 mV. A, top: typical current traces in a control neuron in low Ca\(^{2+}\) (0.5 mM) solution with TTX. Bottom: typical current traces in a kindled neuron in low Ca\(^{2+}\) solution with TTX. B, amplitude distribution histograms for 6 control (●) and 6 kindled (★) neurons in low Ca\(^{2+}\) solution with TTX. C: normalized cumulative amplitude distributions for control (●●●●) and kindled (♦♦♦♦) neurons. Data shown in B were used. D: normalized cumulative interevent interval distributions for control (●●●●) and kindled (♦♦♦♦) neurons. Note that the mean amplitude was significantly increased and the interevent interval was significantly shortened in kindled neurons (P < 0.01 by Kolmogorov-Smirnov test). Data are taken from 40 s of recordings in control and kindled neurons (n = 6 in each case) with 1,278 events for the control distribution and 1,298 events for the distribution histograms of kindled neurons.

synapses increases at the proximal dendrite and/or the soma in kindled neurons.

All these results suggest that both the enhancement of the amplitude and the shortening of the interevent interval of spontaneous CNQX-sensitive EPSCs in kindled BLA neurons are probably due to nearly synchronous multiquantal events resulted from the increased Glu release probability at the nerve terminals. The rise time of evoked CNQX- and AP5-sensitive EPSCs and the decay time constant of evoked CNQX-sensitive EPSCs were shortened. These results suggest that excitatory synapses at the proximal dendrite and/or the soma in kindled neurons may contribute more effectively to generate evoked EPSCs than those at distal dendrites. This augmentation of evoked CNQX- and AP5-sensitive EPSCs may contribute to the epileptiform discharges in kindled neurons.

We thank Dr. J. Nakamura for expert instruction in the operation for kindling rats. We also thank Prof. E. M. McLachlan and Dr. S.M.C. Cunningham for valuable comments and for editing this manuscript.

This work was supported in part by a Grant-in-Aid for Scientific Research of Japan and an Ishibashi Foundation Grant.

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Received 10 November 1997; accepted in final form 16 April 1998.

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