EEG spikes and thereto corresponding hyperpolarizations of pyramidal cells in the hippocampus of the normal rabbit

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Summary
1) EEG spikes were studied in the hippocampus of normal and septum-lesioned rabbits in acute experiments. They were anesthetized with Nembutal and immobilized with D-tubocurarine.

2) EEG spikes recorded with concentric electrodes occurred spontaneously in normal as well as septum-lesioned rabbits. They disappeared after the mid-pontine pretrigeminal transection in both preparations.

3) EEG spikes were recorded also with the microelectrode. This indicates that EEG spike generation was not subsequent to injury discharge due to gross electrode introduction.

4) The intracellular counterpart of the EEG spike was a hyperpolarization of the pyramidal cell, which consisted of Cl-dependent and Cl-non-dependent components. Their magnitudes were much smaller than those of the interictal spike.

Key words: rabbits, hippocampus, electroencephalographic spikes, theta rhythm, inhibitory postsynaptic potential

Introduction
It is generally agreed that EEG spikes indicate the presence of pathological changes in the brain. There are, however, a few articles which describe the existence of EEG spikes in the normal hippocampus. Namely, Imamura & Kawamura and Vanderwolf et al. reported that EEG spikes appeared when the activity level of the hippocampus shifted toward sleep or a lower level. Take observed in the chronic experiments that the EEG spikes diminished completely after the mid-pontine pretrigeminal transection.

On the other hand, Fujita et al. revealed that the intracellular correlate of the normal EEG spike was the hyperpolarization of the hippocampal pyramidal cells. The similar results were obtained in the interictal spikes in the kindled hippocampus.

The purpose of the present experiments is to confirm the following items: (1) whether there are any alterations in generation of EEG spikes in either normal or septum-lesioned rabbits after mid-pontine pretrigeminal transection, (2) whether EEG spikes can be recorded in the hippocampus with thin glass microelectrodes, and (3) what is the intracellular correlates corresponding to EEG spikes in the normal hippocampus.

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Materials and Methods

Thirty-two rabbits (1.0–4.5 kg) were used. Of these, 15 rabbits were considered as "normal" rabbits, because the existence of any diseases was not demonstrated, nor were any artifacts, such as electrode implantation, added. In 14 rabbits, the septum was electrolytically destroyed within 3 days after birth.

Acute experiment

Five rabbits were employed for recording EEGs in the hippocampus whereas 10 for intracellular recording of hippocampal pyramidal cells. They were anesthetized with pentobarbital sodium (Nembutal, 20–30 mg/kg, i.v.) and immobilized with D-tubocurarine (Amerizol, 2–3 mg/kg, i.v. and i.m.).

For introduction of microelectrodes, two holes of about 5 mm in diameter were made symmetrically in both parietal bones. A small hole of about 2 mm in diameter was also made in the bregma for introduction of a concentric electrode into the fornix. The hippocampi were not exposed in order to avoid possible injuries inflicted upon. But the dura mater was cut open. The exposed neocortical surface was covered with 2% Ringer-agar solution. An Ag-AgCl indifferent electrode (a silver plate) was placed on the frontal bone. The glass microelectrodes were filled with either 2 M NaCl solution containing fast green, 3 M KCl solution, or 2 M potassium citrate solution. The last two were used for intracellular recording. The tips of all of these electrodes were artificially broken so as to reduce the resistance. After the tips were broken, the resistance of the electrodes filled with 3 M KCl or 2 M K-citrate ranged from 5 to 15 MΩ. Preamplifier (WPI 701) was employed for observing intracellular potentials, together with a dualbeam cathode ray oscillograph (Sony-Tektronix R 5103). A polygraph (Nihonkoden, RM 80) was used for recording both hippocampal EEGs and intracellular potentials.

For monitoring EEGs, a concentric electrode, insulated except at the tips and about 750 μm in diameter, was introduced into the right hippocampus. The concentric electrode was advanced until a seizure discharge was therewith induced in the hippocampus. The electrode was further advanced by about 500 μm and fixed there.

When EEGs were studied with glass microelectrodes filled with 2 M NaCl, they were introduced into both hippocampi. Their arrival in the hippocampus was signalled by the appearance of the theta rhythm. After confirming the existence of "null zone", the electrodes were further advanced by approximately 500 μm and fixed there.

Either the fornix or the left hippocampus was stimulated through a concentric electrode. An electronic stimulator (Nihonkoden SEN 3201), together with an isolation unit, was employed for the fornical and the hippocampal stimulation. A single rectangular pulse of 1 msec in duration was delivered supramaximally (above 60 mV).

After the experiments, a d.c. (100 μA, 5–10 min) was passed through the microelectrode to locate the tip of the NaCl-filled electrode. Histological examination was performed with the Nissl stain.

Mid-pontine pretrigeminal transection.
Mid-pontine pretrigeminal transection was performed on twenty six rabbits. The transection was made with a spatula, which was introduced into the brainstem at an angle of about 40 degrees from the frontal plane. The level of the section was confirmed under direct vision after the experiments.

Identification of pyramidal cells

All the cells described below will be referred to as pyramidal cells, inasmuch as they exhibited a part or all of the following electrical activities, i.e., a large inhibitory postsynaptic potential (IPSP) and/or current-induced inactivation response, i.e., a spike burst with a large underlying depolarization (Kandel and Spencer, Fujita and Sakuranaga, Fujita and Iwasa).

Results

EEG spikes in the normal hippocampus

In Fig. 1, EEGs were observed with the concentric electrode. When the theta rhythm was irregular in pattern and low in amplitude, EEG spikes appeared (Fig. 1 A). Their amplitude was 2 mV at the most. After the pretrigeminal transection, the EEGs exhibited a continuous theta rhythm and EEG spikes vanished completely (Fig. 1 B). In the rabbit with a complete septal lesion, there was absolutely no theta rhythm, but fast wave activity dominated together with interspersed EEG spikes (Fig. 1 C). The magnitude of EEG

![Fig. 1 Hippocampal EEGs in acute preparation of a normal rabbit (A and B) and a rabbit with complete septal lesion (C and D), which was carried out in its neonatal period (within 3 days after birth). Before (A and C) and after (B and D) pretrigeminal transection. Regardless of whether the septum was destroyed or not, EEG spikes occurred spontaneously. However, pretrigeminal transection made them disappear completely. In D, no hippocampal theta rhythm was observed whatsoever during the experiment. Calibrations in B and D apply to A and C.](image-url)
spikes was about 2 mV. After the pretrigeminal transection the EEGs became a low voltage fast activity, and EEG spikes disappeared (Fig. 1 D).

EEGs were then observed with 2 M NaCl-filled glass microelectrodes, which did not generate any injury discharges upon introduction. EEG spikes appeared spontaneously (Fig. 2), when the theta rhythm was absent. They were about 1 mV in magnitude, and disappeared when the theta rhythm appeared. EEG spikes in both hippocampi could be synchronous (Fig. 2 B and C) or could appear only in one hippocampus (Fig. 2 A and D).

Thus EEG spikes were recorded with both gross and microelectrodes. This indicates that the EEG spikes were not triggered by the injury discharges which usually appeared on the introduction of the gross electrode. However, there was a tendency that the magnitude of the EEG spike recorded with concentric electrode was larger than that recorded with microelectrode. The difference in the amplitude suggests the possible presence of the “acute kindling” effect\(^{11}\) (see Discussion).

**Intracellular recording from pyramidal cells in the normal hippocampus**

Eighty-nine cells were successfully impaled. When recorded with K-citrate electrodes (8 cells), intracellular counterparts corresponding to EEG spikes were hyperpolarizations without any preceding depolarizations (Fig. 3 A, B and C, dots). The magnitude of the hyperpolarizations was less than 10 mV. The inactivation response (IR), i.e. a large, prolonged depolarization with a superposed spike burst\(^{11}\), and spikes were also observed in the same cell. There were, however, no particular EEG patterns corresponding to the inactivation response. When recorded with KCI electrodes (19 cells), spontaneous potentials synchronous with EEG spikes were diphasic potentials, i.e. early depolarizing and late hyperpolarizing potentials (Fig. 4 A, B and C, dots). Supramaximal stimulation of the fornix (upward arrow in Fig. 4 A) induced an antidromic spike followed by a similar diphasic potential. These results indicate that the early depolarization observed with the KCI electrode is nothing but Cl-dependent hyperpolarization. Consequently, the hyperpolarization correspo-
nding to the EEG, spike consisted of two components, that is, Cl-dependent and Clnon-dependent components. Hyperpolarization was never preceded by a spike burst, nor by any depolarization shifts (Fig. 3A, B and C, dots).

In general, EEG spikes were fairly well correlated to intracellular hyperpolarizations with respect to time, but variable with respect to shape and magnitude. Hyperpolarizations could occur without corresponding EEG spikes (Fig. 5 A).

In some cells spike discharges did occur synchronously with EEG spike (Fig. 5 B). Thus there seem to be two kinds of EEG spikes. One is those corresponding to depolarization and the other those corresponding to hyperpolarizations. They can be conveniently referred to as excitatory and inhibitory EEG spikes. This is consistent with previous reports.

**Discussion**

The present experiments have shown that the EEG spike appears in the hippocampus even when the generation of seizure discharge was avoided upon introduction of the gross electrode. This proves that seizure discharge is not the prerequisite for generation of the EEG spikes in the normal hippocampus. According to the recent studies, however, "acute kindling" can be produced in a brief time span even in acute experiments, and electrolytic destruction of the entorhinal cortex consistently produces limbic seizure activity. In fact, the amplitude of the EEG spike recorded with the gross electrode (Fig. 2 A) was larger than those with the glass microelectrode (Fig. 3 A and B), being 2 mV and 1 mV respectively. This would be of significance, because it is the microelectrode that should register a greater current density. Consequently, it is suggested that the introduction of the gross electrode may result in acute kindling.

EEG spikes appeared when the theta rhythm was absent. This is compatible with the report that EEG spikes in the hippocampus were observed at a lower activity level. Moreover, both normal and interictal spikes disappeared after the mid-pontine pretrigeminal transection. This phenomenon was observed even in the rabbits whose septum had been completely electrolyzed within the first 3 days after birth, and therefore no theta rhythm appeared under any circumstances. It is therefore concluded that theta wave itself was not responsible for the suppression of the EEG spikes.
Fig. 4  Upper records: intracellular records obtained from a pyramidal cell in CA 3-4 region with the K-Cl electrode. Lower traces: hippocampal EEGs. Spontaneously occurring normal EEG spikes and their intracellular correlates of the hippocampal pyramidal cell (A-C, dots) were recorded. At an arrow, the fornix was stimulated supramaximally. Note that the early parts of the spontaneous and fornix-induced hyperpolarizations were converted to depolarizing potentials. The early-depolarizing potential is presumably due to Cl diffusion. Calibrations in A apply to B and C.

Fig. 5  Upper traces: intracellular records obtained from a pyramidal cell in CA 3-4 region with the K-citrate electrode. Lower traces: hippocampal EEGs. In A, spontaneous hyperpolarizations did not correspond to EEG spikes (A, dots). In B, spike discharges were recorded synchronously with the EEG spikes (B, dot).
Another salient fact was that the intracellular counterpart of the normal EEG spike was a pure hyperpolarization consisting of both the Cl-dependent and Cl-non-dependent components, which was not preceded by a spike burst. Dichter and Spencer reported that the penicillin-induced focus was characterized by the presence of a spike burst with underlying large depolarization. The normal EEG spike is obviously different in generating mechanism from the penicillin focus. On the other hand, Fujita and Sakuranaga demonstrated that the EEG spike produced in the hippocampus through a kindling procedure could be an extracellular counterpart of pure hyperpolarization of pyramidal cells. The hyperpolarization was never preceded by any spike burst. Furthermore, it was revealed that it consisted of both the Cl-dependent and Cl-non-dependent components. It is known that the inhibitory postsynaptic potential originating in the soma is reversed to a depolarizing potential when the intracellular recording is performed with the KCl electrode. In the present experiments, it was confirmed that the early depolarizing potential was due to the Cl diffusion as explained by Fujita. Therefore, it can be presumed that the early depolarization is the somatic IPSP.

From the point of view of the intracellular events, the normal EEG spike observed in the present experiments is thought to be identical with the interictal spike, and the difference between them is only quantitative, because the normal EEG spike and its intracellular counterpart were much smaller than the interictal spike and its intracellular counterpart, respectively (cf. Fujita and Sakuranaga).

It was demonstrated in the present experiments, that there were two types of normal EEG spikes, although the excitatory type was not fully investigated in the present experiments. The recent studies showed that the frequency of interictal EEG spikes correlates with decreased seizure susceptibility, and that the intracellular counterpart of the interictal spike is a hyperpolarization of the hippocampal pyramidal cell. Fujita et al. proved that the enhancement of inhibition was one of the characteristics of the kindled hippocampus. In the present experiments, it is strongly suggested that the EEG spike corresponding to the hyperpolarization has the same inhibitory function as those of the interictal spike.

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References

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