Title

Decrease in Synaptic Transmission Can Reverse the Propagation Direction of Epileptiform Activity in Hippocampus in vivo

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Propagation Direction of Epileptiform Activity in vivo

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ABSTRACT

Most types of epileptiform activity with synaptic transmission have been shown to propagate from the CA3 to CA1 region in hippocampus. However, non-synaptic epileptiform activity induced \textit{in vitro} is known to propagate slowly from the caudal end of CA1 toward CA2/CA3. Understanding the propagation modes of epileptiform activity and their causality is important to revealing the underlying mechanisms of epilepsy and developing new treatments. In this paper, the effect of the synaptic transmission suppression on the propagation of epilepsy \textit{in vivo} was investigated by using multiple-channel recording probes in CA1. Non-synaptic epileptiform activity was induced by calcium chelator EGTA with varied concentrations of potassium. For comparison, dis-inhibition synaptic epileptiform activity was induced by picrotoxin (PTX) with or without partial suppression of excitatory synaptic transmission. The propagation velocity was calculated by measuring the time delay between two electrodes separated by a known distance. The results show that \textit{in vivo} non-synaptic epileptiform activity propagates with a direction and velocity comparable to those observed in \textit{in vitro} preparations. The direction of propagation for non-synaptic activity is reversed from the PTX induced synaptic activity. A reversal in propagation direction and change in velocity were also observed dynamically during the process of synaptic transmission suppression. Even a partial suppression of synaptic transmission was sufficient to significantly change the propagation direction and velocity of epileptiform activity. These results suggest the possibility that the measurement of propagation can provide important information about the synaptic mechanism underlying epileptic activity.
INTRODUCTION

Many types of epileptiform activity, such as partial seizures, are known to initiate from a primary focus and then propagate to other brain regions (McCormick and Contreras, 2001). Therefore, understanding the propagation pathways of epileptiform activity can be helpful to reveal the mechanisms of epilepsy and develop new treatments. The hippocampus is one of the most epilepsy prone brain regions. Many types of epileptiform activity involving synaptic transmission have been observed to propagate from CA3 region to CA1 in hippocampus, such as the interictal burst induced in high K+ solution (Korn et al. 1987); the epileptiform discharge induced by zero-Mg2+ solution (Kohling et al 2001); the afterdischarge evoked by stimulus trains (Rafiq et al 1993); and the bursts caused by convulsant drugs such as picrotoxin, 4-aminopyridine or pilocarpine (Hablitz 1984; Luhmann et al 2000, Nagao et al 1996). However, it has been observed in vitro that the non-synaptic epileptiform activity induced in low-Ca2+ solution with high K+ propagates from the caudal end of the CA1 to the CA3 with a slow velocity (Haas and Jefferys 1984; Yaari et al 1983 and 1986; Lian et al 2001).

Since the non-synaptic epilepsy model was established in hippocampal slices in 1980s (Jefferys and Haas 1982; Taylor and Dudek 1982; Konnerth et al. 1984), non-synaptic epileptiform activity had not been observed in vivo until recently (Feng and Durand, 2003). Yet, little is known about the propagation of this type of epileptiform activity in vivo. In this paper we address the two following questions: Does non-synaptic epileptiform activity induced in vivo propagate in the similar manner as in vitro? How does suppression of synaptic transmission modulate the propagation direction and velocity during already induced
epileptiform activity? These questions were investigated by inserting multiple-channel recording probes into an exposed rat hippocampus \textit{in vivo}. Several different types of non-synaptic and synaptic epileptiform activity were induced by calcium chelator (EGTA) or by GABAergic blocker picrotoxin (PTX). The relationship between synaptic transmission suppression and changes in both propagation direction and velocity of epileptiform activity was also observed dynamically.
METHODS

Surgical procedures

All procedures used in this study were approved by the Institutional Animal Care and Use Committee, Case Western Reserve University, Cleveland. Adult Sprague Dawley rats (260 - 360 g, n = 21) were anesthetized with Urethane (1.5 g/kg, i.p.) and placed in a stereotaxic apparatus. Body temperature was maintained at 37 °C with a heating pad. The skull over the left cortex was opened and the cortex overlying the left dorsal hippocampus was removed. Artificial cerebrospinal fluid (ACSF) solution was warmed to 37 °C and placed over the surface of the exposed dorsal hippocampus. The solution was refreshed every 5 min throughout the experiment by sucking away old solution and dropping in fresh solution using syringes (Feng and Durand, 2003).

Solutions and Drugs

Normal ACSF consisted of (in mM): 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1.5 MgSO₄, 26 NaHCO₃ and 2 g/L D-glucose. Calcium chelator ethylene glycol-bis (β-aminoethyl ether) -N,N,N',N'-tetraacetic acid (EGTA) 5 mM replaced CaCl₂ to lower [Ca²⁺]₀ and block synaptic transmission in hippocampus to induce non-synaptic epileptiform activity in vivo with 7.5 or 12 mM KCl. ACSF without CaCl₂ was used to partially block synaptic transmission in the in vivo preparation. Because of the difficulty in washing out [Ca²⁺]₀ from the hippocampal tissue in this in vivo preparation, zero Ca²⁺ solution can only partially block synaptic transmission. Picrotoxin (PTX, 0.1 - 0.4 mM) was used to induce epileptic bursts by blocking the GABAₐ receptor. All chemicals were obtained from Sigma or
Fisher.

**Recording of spontaneous and evoked potentials**

Multi-channel silicon recording probes with two shanks provided by the Center of Neural Communication Technology, University of Michigan, were used for recording. The distance between the two shanks was 600 µm with 4 electrodes distributed in each shank at a distance of 200 µm. Bipolar stimulating electrodes were made from pairs of insulated nichrome wires (80 µm in diameter) with a 0.5 mm vertical tip separation. Recording probes were positioned in the exposed left hippocampus (AP –3.0, ML 2.6) to record field potentials in the CA1 pyramidal stratum and in the CA1 stratum radiatum. Stimulation electrodes were inserted into the area (AP –2.0, ML 2.3) for stimulating the Schaffer collaterals. Patterns of the evoked potentials guided vertical positioning of both the recording probes and the stimulation electrodes (Kloosterman et al. 2001). The stimulus was 0.1 ms duration with 0.35 mA constant current. Two stainless steel screws fixed in the bone of the nose served as ground and reference electrodes.

CA1 field potential signals were amplified 1000 times by two Model 1700 4-channel amplifiers (A-M System Inc.) with filter frequency ranges from 0.1 Hz to 5 KHz for both spontaneous and evoked potentials. Signals were then sampled at a rate of 20 KHz by using a ML795 PowerLab/16SP data acquisition system (ADInstruments) before they were stored into a hard disk for off-line analysis. The amplitude of orthodromic evoked population spikes (PS) and the PS latency were calculated from the pyramidal layer recordings to evaluate the change in synaptic transmission.
The time delay between signals obtained from two recording shanks was calculated by
the latency difference between the most negative points of the spikes or waves recorded from
the pyramidal layer. Signals in the pyramidal layer, especially those with spike activity, have
more abrupt slopes facilitating the measurement of the delay. The propagation velocity was
calculated by dividing the distance (0.6 mm) between two electrodes by the delay. Data are
expressed as mean ± standard deviation. A student's t-test was used for statistical analysis.
RESULTS

Propagation difference between non-synaptic and synaptic epileptiform activities

Figure 1 shows the placements of a recording probe and a stimulating electrode (S1) in the CA1 of an exposed left hippocampus. The recording probe included two shanks (R1 and R2) each having 4 electrode contacts. The two shanks were placed parallel with the middle line of the brain in a presumably transverse plane of the hippocampus. R1 is located near the CA3 region and R2 is near the subiculum. Two of the 4 contacts in each shank were used to record extracellular field potentials from pyramidal layer and from stratum radiatum, respectively. About 30 min following the application of ACSF solution with 7.5 mM K⁺ and 5 mM EGTA over the exposed hippocampus, slow-wave activity with or without superimposed spikes appeared when synaptic transmission was depressed as a result of lowering [Ca²⁺]₀ (Fig. 2A and 2B). This activity was similar to that previously reported (Feng and Durand, 2003). The suppression of synaptic transmission was clearly indicated by the disappearance of orthodromically evoked PS in the pyramidal layer. The slow-waves in the pyramidal layer were out of phase with those in the stratum radiatum and propagated from R2 to R1 (Fig. 2A). Slow-waves with superimposed spikes usually propagated faster with a shorter delay between R2 and R1 than the slow-waves without superimposed spikes (Fig. 2B). Figure 2C shows a histogram of slow-wave delay calculated from 155 slow-waves without superimposed spikes collected within a same preparation. The delay was measured from pyramidal layer field potentials recorded by R1 and R2, as illustrated in Figures 2A. The delays varied significantly (–187 ± 158 ms/0.6
mm). The negative value indicates propagation from R2 to R1. Figure 2D shows a delay histogram of slow-wave calculated from 172 slow-waves with superimposed spikes in another preparation. The mean delay was \(-33 \pm 39 \text{ ms/0.6 mm}\).

However, for epileptiform activity induced by the GABAergic blocker PTX with intact excitatory synaptic transmission, the propagation was different both in direction and velocity (Fig. 3). The application of an ACSF solution containing PTX 0.1 – 0.4 mM (7 mM K\(^+\), 2 mM Ca\(^{2+}\)) over the exposed hippocampus induced interictal activity or periodical bursts (Fig 3A, middle). The orthodromic evoked response displayed multiple PSs resulting from a loss of GABA\(_A\) inhibitory synaptic transmission (Fig 3A, left). The spikes in PTX induced bursts propagated from R1 to R2 (expansions in Fig. 3A). Figure 3B shows a histogram of spike delay between R1 and R2 measured from 464 spikes collected in a single preparation. All of the delays were positive indicating propagation from R1 to R2.

The average delay of the PTX induced spikes was \(1.4 \pm 0.53 \text{ ms/0.6 mm (n = 4)}\) equal to an average velocity of \(472 \pm 150 \text{ mm/s}\), while the average delay of non-synaptic slow-waves induced by EGTA was \(-177 \pm 122 \text{ ms/0.6 mm (n = 4)}\) equal to an average velocity of \(-11 \pm 15 \text{ mm/s}\) (Table 1, group 1). The delays were significantly different between the two groups (\(P < 0.05\)).

These results show that the block of synaptic transmission by lowering \([\text{Ca}^{2+}]_o\) can change both the propagation direction and velocity of epileptiform activity. Next, the effect of synaptic transmission suppression on the propagation changes was tested dynamically in the same experimental preparation.
Dynamic propagation changes in epileptiform activity

By perfusing an ASCF solution with high K⁺ (12 mM), zero Ca²⁺ and 5 mM EGTA over the exposed hippocampus, a novel epileptic model characterized by sustained spike activity was observed. Potassium (K⁺) 12 mM was used for about 40 min to raise the neuronal excitability without any epileptiform activity. Following the application of EGTA, epileptiform activity started several minutes later as synaptic transmission had not yet been completely abolished. The activity lasted for more than one hour surviving the complete block of synaptic transmission (Fig. 4A). During the early period of spike activity (termed as early-spike) with partial synaptic transmission, as indicated by large evoked PS, spikes propagated from R1 to R2 (Fig. 4A, left). However, with synaptic transmission being gradually blocked, as indicated by the amplitude decrease of evoked PS until a total disappearance, the spikes in spontaneous potential appeared as sustained 3 – 5 Hz paired-spikes (termed as late-spike). Their propagation direction was from R2 to R1 (Fig. 4A, middle and right). Figure 4B shows the changes in spike delay due to suppression of synaptic transmission for an 80 min period of 12 mM K⁺ with 5 mM EGTA. For multiple spikes, the delay was always measured from the first spike. During the process of synaptic transmission suppression, the delay changed from small positive values for early-spikes to large negative values for late-spikes. Statistical analysis (Table 1, group 2) shows that the delay for early-spikes (0.73 ± 0.96 ms/0.6 mm, a velocity of 742 ± 300 mm/s) is significantly different from the delay for late-spikes (−4.4 ± 2.3 ms/0.6 mm, a velocity of 192 ± 147 mm/s; P<0.01; n = 9). This result shows that the propagation changes induced by the block of synaptic transmission could be observed directly in a single preparation. A similar change was also
observed in the propagation of PTX induced epileptiform activity during partial suppression of synaptic transmission.

In this PTX experimental group (n = 4), ACSF with 12 mM K⁺ and Ca²⁺ free was first perfused over the exposed hippocampus for more than 40 min to lower CA1 [Ca²⁺]o to partially suppress the synaptic transmission, as indicated by a significant increase of PS-latency in evoked potentials. The amplitude increase of the PS was due to the increase of neuronal excitability by higher K⁺ and lower Ca²⁺ concentrations. PTX (0.1 mM) was then added to the solution to evoke epileptiform activity. This activity propagated from R2 to R1 (Fig. 5A). Following the addition of 2 mM Ca²⁺ to the perfusion solution, the pattern of spontaneous epileptiform activity changed and it propagated in a reversed direction from R1 to R2 (Fig. 5B). Statistical analysis (Table 1, group 3) shows that during the Ca²⁺ free period the propagation delay of –8.1 ± 6.0 ms/0.6 mm (a velocity of -131 ± 107 mm/s) was significantly different from the propagation delay of 1.9 ± 0.44 ms/0.6 mm (a velocity of 361 ± 87 mm/s) during the 2 mM Ca²⁺ period (P < 0.01; n = 4). This result shows that even a partial block of synaptic transmission is sufficient to significantly change both the direction and velocity of PTX induced epileptiform activity.
DISCUSSION

The main findings of this study are: (1) Non-synaptic epileptiform activity induced \textit{in vivo} in the CA1 region propagates from caudal CA1 to rostral CA1, a reverse direction from PTX induced synaptic epileptiform activity. The propagation velocity of the non-synaptic epileptiform activity was also significantly lower than PTX induced epileptiform activity. (2) Suppression of excitatory synaptic transmission can change both the propagating direction and velocity of epileptiform activity.

\textit{Propagation of non-synaptic activity in \textit{in vivo} and \textit{in vitro} preparations}

Two types of non-synaptic epileptiform activity were observed in this study: (1) slow-wave with or without superimposed spikes induced by 7.5 mM K\(^+\) and 5 mM EGTA; (2) sustained 3–5 Hz paired-spikes (late-spike) induced by 12 mM K\(^+\) and 5 mM EGTA. Both types of activity propagated from caudal end of CA1 to the rostral CA1 in the same hippocampus as the non-synaptic activity \textit{in vitro} (Haas and Jefferys 1984; Yaari et al. 1983 and 1986; Lian et al. 2001). They are discussed separately below:

The slow-wave induced by EGTA is similar to the non-synaptic depolarization burst induced by low-Ca\(^{2+}\) \textit{in vitro} (Jefferys and Haas 1982; Taylor and Dudek 1982; Konnerth et al. 1984). Since it is difficult to wash out \([\text{Ca}^{2+}]_o\) from hippocampal tissue \textit{in vivo}, adding the calcium chelator EGTA is necessary to lower \([\text{Ca}^{2+}]_o\) and induce non-synaptic epileptiform activity. The average propagation velocity of slow-waves (-11 ± 15 mm/s) \textit{in vivo} is within the range of propagation velocities observed \textit{in vitro}, 1 – 100 mm/s (Haas and Jefferys 1984) or near 0.44 – 4.2 mm/s (Konnerth et al. 1986). Also, the propagation velocity is as variable \textit{in
vivo as it is in vitro (Haas and Jefferys 1984; Konnerth et al. 1986).

The late-spikes propagate faster (-192 ± 147 mm/s) than the in vitro non-synaptic bursts (1 – 100 mm/s). Several reasons might account for this difference. (1) The propagation velocity of the non-synaptic burst is [K+]o dependent and increases markedly with increasing [K+]o in vitro (Yaari et al 1986). We used a solution with a high concentration of 12 mM K+ thereby explaining the higher propagation speed. (2) In vitro, non-synaptic epileptiform activity with more abrupt onsets (spike-like activity) was also observed to propagate faster with a velocity about 100 mm/s in vitro (Haas and Jefferys 1984; Jefferys 1995).

Another type of potential waveform propagating slowly with non-synaptic mechanisms observed in brain tissue is spreading depression (SD; Somjen 2001; Somjen et al 1992). SD is very different from the low-calcium non-synaptic epileptiform activity reported here in vivo and in vitro. SD is characterized by larger and longer depolarization potential shift preventing neuronal firing and a much lower propagation velocity in the range of a few millimeters per minute. The mechanisms involve the diffusion of either K⁺ or glutamate, (Somjen 2001).

Suppression of synaptic transmission change the propagation of epileptiform activity

The significant differences in the propagation delays amongst the three paired groups in Table 1 show that the suppression of synaptic transmission changed the propagation direction and lowered propagation velocity. This result was not only observed in the first group between the fully developed non-synaptic activity (slow-wave) and synaptic activity (PTX-induced-spike), but also was shown by the dynamic propagation evolution recorded during EGTA induced changes in synaptic transmission (group 2). Moreover, it was also
observed with partial depression of synaptic transmission in PTX induced epileptiform activity (group 3). This result is consistent with previous in vitro studies showing that suppression of excitatory synaptic transmission can decrease the propagation velocity of dis-inhibited activity in the brain slices (Golomb and Amitai 1997; Traub et al. 1993).

However, it is interesting that the suppression of synaptic transmission can also change the propagating direction. Interictal synaptic activity usually initiates in CA3 and propagates to CA1 (McNamara 1994; Luhmann et al. 2000; Kohling et al. 2001). Ictal activity was observed originating in the entorhinal cortex and propagating via dentate gyrus to CA3, then to CA1 (Nagao et al. 1996; Barbarosie and Avoli 1997; Dzhala and Staley 2003). Therefore, most types of epileptiform activity propagate in the direction from CA3 to CA1. Nevertheless, in isolated subiculum-CA1 slices, spontaneous activity induced by low magnesium media was also found propagating from subiculum to CA1 and spreading from the caudal to the rostral part of CA1 (Harris and Stewart 2001). One possible mechanism for this phenomenon is that the subiculum generates spontaneous epileptiform activity that then propagates into CA1 through a backward projection from subiculum to CA1 region when the projection from the CA3 to CA1 is eliminated (Behr and Heinemann 1996). However, it seems unlikely that this mechanism could account for the change in propagation direction observed in the present study, since the low-Ca²⁺ would suppress the synaptic connections in the CA1 region projecting from both the CA3 and subiculum. Moreover, non-synaptic activity has been shown to spread through potassium wave diffusion, a non-synaptic mechanism (Konnerth et al. 1986; Lian et al 2001). An alternative explanation for the change in propagation direction is based on the fact that the low-Ca²⁺ non-synaptic epileptiform activity is more readily
evoked in the CA1 region than in both the CA3 and dentate gyrus (Snow and Dudek 1984; Konnerth et al. 1984; Schweitzer et al. 1992). The more densely packed pyramidal cells in the CA1 region may facilitate non-synaptic processes such as gap junctions, ephaptic interaction and potassium coupling (Jefferys 1995; Konnerth et al. 1984). Therefore, the non-synaptic epileptiform activity might tend to invade into CA3 from the original CA1 area resulting in a propagation direction from CA1 to CA3.

**Conclusion**

The results of this study show, for the first time, that the non-synaptic epileptiform activity *in vivo* propagates slowly in a direction from caudal CA1 toward the CA3. It is also clearly shown that the suppression of excitatory synaptic transmission can change both the propagation direction and velocity of an epileptiform activity. These results suggest that the synaptic and non-synaptic epilepsy propagate in different modes, implying that measuring the propagation of seizure activity in the brain could provide important information about the synaptic mechanisms underlying epilepsy.
We thank Alicia Jensen for editorial assistance.

DISCLOSURES

This work was supported by the National Institute of Neurological Disorders and Stroke Grant RO1 NS-40785. The recording probes were provided by the University of Michigan Center for Neural Communication Technology sponsored by NIH/NCRR grant P41 RR09754.
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Snow RW and Dudek FE. Synchronous epileptiform bursts without chemical transmission in CA2, CA3 and dentate areas of the hippocampus. Brain Res 298: 382-385, 1984.


Table 1 Effect of synaptic transmission suppression on propagation direction and velocity

<table>
<thead>
<tr>
<th>Paired group</th>
<th>Activity with little or no suppression of excitatory synaptic transmission</th>
<th>Activity with significant suppression of excitatory synaptic transmission</th>
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<tbody>
<tr>
<td></td>
<td>Activity name</td>
<td>Delay (ms/0.6mm)</td>
</tr>
<tr>
<td>1</td>
<td>PTX-spike (Ca²⁺ 2 mM)</td>
<td>1.4 ± 0.53</td>
</tr>
<tr>
<td></td>
<td># (n = 4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Early-spike (EGTA 5 mM)</td>
<td>0.73 ± 0.96</td>
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<tr>
<td></td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PTX-spike (Ca²⁺ 2 mM)</td>
<td>1.9 ± 0.44</td>
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<tr>
<td></td>
<td>(n = 4)</td>
<td></td>
</tr>
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</table>

Negative delay or velocity indicate activity propagating from the caudal CA1 to the rostral CA1.

* The number of experiments.

* P < 0.05; ** P < 0.01 activity with little suppression of excitatory synaptic transmission vs. activity with significant suppression of excitatory synaptic transmission, student's t-test.
Figure Legends

Fig. 1 Schematic diagram of the experimental preparation and the locations of electrodes in CA1. The recording probe included two shanks (R1 and R2) with a distance of 0.6 mm. Two contacts in each shank were used to collect extracellular field potentials from the pyramidal layer and stratum radiatum, respectively. The stimulating electrode (S1) was placed to orthodromically stimulate the Schaffer collaterals.

Fig. 2 Propagation of low-Ca\(^{2+}\) epileptiform activity in CA1 in-vivo. A: Propagation of slow-waves without superimposed spikes. 30 min following the application of a solution with K\(^+\) 7.5 mM and EGTA 5 mM, the synaptic transmission was blocked, indicated by no PS in orthodromic evoked potential in pyramidal layer compared to baseline recording (left). Slow-waves in the spontaneous potentials propagated from R2 to R1 in both pyramidal layer and stratum radiatum (right). B: Propagation of slow-waves with superimposed spikes in another experiment. Slow-waves were evoked by a solution of K\(^+\) 7.5 mM and EGTA 5 mM while synaptic transmission was blocked, indicated by evoked potentials without PSs in pyramidal layer (only potentials in pyramidal layer were shown). They propagated from R2 to R1, as illustrated by three expanded portions of the waves. C: Delay histogram of slow-waves without superimposed spikes between R1 and R2 measured from 155 waves of an experiment. D: Delay histogram of slow-waves with superimposed spikes between R1 and R2 measured from 172 waves of an experiment. Negative value means propagation from R2 to R1.

Fig. 3 Propagation of the epileptiform activity induced by PTX 0.4 mM in the CA1 pyramidal
layer. A: Large multiple PSs in the evoked potential indicated the decrease of inhibition activity by PTX (left). Spikes in a spontaneous potential burst propagated from R1 to R2 (middle) illustrated by the two expansions (right). B: Histogram of spike delays (ms/0.6 mm) between R1 and R2 measured from 464 spikes from a single experiment. Positive value means propagation from R1 to R2.

Fig. 4 Propagation changes in the epileptiform activity evoked by high-K⁺ (12 mM) with 5 mM EGTA. A: Spontaneous epileptiform activity in pyramidal layer recorded following 3, 40 and 70 min of EGTA application with evoked potential in the left corner. Time scale expansion of the spikes illustrated the change in spike propagation from R1 to R2 into R2 to R1. B: Change of spike delay due to the suppression of synaptic transmission indicated by the decrease in amplitude of orthodromically evoked PS for the 80 min period. Positive delay value means propagation from R1 to R2. During the early period of spike activity when synaptic transmission had not yet been blocked, spikes propagated from R1 to R2. However, when synaptic transmission was later blocked, the spikes propagated from R2 to R1 with a slower velocity.

Fig. 5 Propagation changes in PTX induced epileptiform activity by partial suppression of the synaptic transmission. Evoked and spontaneous potentials in pyramidal layer recorded by R1 and R2. A: Adding PTX 0.1 mM in ACSF with K12 mM and Ca²⁺ free evoked epileptiform activity when excitatory synaptic transmission was partially blocked by the lowering of [Ca²⁺]o. The activity propagated from R2 to R1. B: 5 min following the addition of Ca²⁺ 2 mM
to the perfusate, the excitatory synaptic transmission recovered, as indicated by the decrease in evoked PS latency and the increase in PS number and amplitude. The shape of spontaneous epileptiform activity changed and its propagation also changed direction, from R1 to R2 with a faster velocity.
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(A) Evoked potential

Baseline

K⁺ 7.5 mM
EGTA 5 mM

Pyramidal layer

Spontaneous potential

(R2: thin line)

R1

R2

Delay

2 mV

5 ms

Stratum radiatum

(B) Evoked potential in pyramidal layer

Baseline

K⁺ 7.5 mM
EGTA 5 mM

Spontaneous potential in pyramidal layer

(R2: dotted line)

R1

R2

1 mV

1 s

Delay

0.05 s

(C) Distribution of delay between R1 and R2 in slow-waves without spikes (%)

Mean = −187 ± 158 ms
Fig. 2 Propagation of low-Ca\(^{2+}\) epileptiform activity in CA1 \textit{in-vivo}. \textit{A}: Propagation of slow-waves without superimposed spikes. 30 min following the application of a solution with K\(^+\) 7.5 mM and EGTA 5 mM, the synaptic transmission was blocked, indicated by no PS in orthodromic evoked potential in pyramidal layer compared to baseline recording (\textit{left}). Slow-waves in the spontaneous potentials propagated from R2 to R1 in both pyramidal layer and stratum radiatum (\textit{right}). \textit{B}: Propagation of slow-waves with superimposed spikes in another experiment. Slow-waves were evoked by a solution of K\(^+\) 7.5 mM and EGTA 5 mM while synaptic transmission was blocked, indicated by evoked potentials without PSs in pyramidal layer (only potentials in pyramidal layer were shown). They propagated from R2 to R1, as illustrated by three expanded portions of the waves. \textit{C}: Delay histogram of slow-waves without superimposed spikes between R1 and R2 measured from 155 waves of an experiment. \textit{D}: Delay histogram of slow-waves with superimposed spikes between R1 and R2 measured from 172 waves of an experiment. Negative value means propagation from R2 to R1.
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