GABA_A Receptor Activity Suppresses the Transition from Inter-ictal to Ictal Epileptiform Discharges in Juvenile Mouse Hippocampus

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Abstract Exploring the transition from inter-ictal to ictal epileptiform discharges (IDs) and how GABA_A receptor-mediated action affects the onset of IDs will enrich our understanding of epileptogenesis and epilepsy treatment. We used Mg²⁺-free artificial cerebrospinal fluid (ACSF) to induce epileptiform discharges in juvenile mouse hippocampal slices and used a micro-electrode array to record the discharges. After the slices were exposed to Mg²⁺-free ACSF for 10 min–20 min, synchronous recurrent seizure-like events were recorded across the slices, and each event evolved from inter-ictal epileptiform discharges (IIDs) to pre-ictal epileptiform discharges (PIDs), and then to IDs. During the transition from IIDs to PIDs, the duration of discharges increased and the inter-discharge interval decreased. After adding 3 μmol/L of the GABA_A receptor agonist muscimol, PIDs and IDs disappeared, and IIDs remained. Further, the application of 10 μmol/L muscimol abolished all the epileptiform discharges. When the GABA_A receptor antagonist bicuculline was applied at 10 μmol/L, IIDs and PIDs disappeared, and IDs remained at decreased intervals. These results indicated that there are dynamic changes in the hippocampal network preceding the onset of IDs, and GABA_A receptor activity suppresses

the transition from IIDs to IDs in juvenile mouse hippocampus.

Keywords Epileptiform discharge · Gamma-aminobutyric acid · Bicuculline · Muscimol · Micro-electrode array · Hippocampal slice

Introduction

An epileptic seizure is a dynamic phenomenon in the brain associated with abnormally excessive or synchronous neuronal activity [1]. In addition to clinical evidence [2, 3], a large number of experimental reports have demonstrated that before the onset of an epileptic seizure, there is a spontaneous transition from brief bursts of inter-ictal epileptiform discharges (IIDs) to more prolonged episodes of ictal epileptiform discharges (IDs) [4–6]. This transition period, which occurs several minutes or even up to hours prior to the onset of a seizure, is recognized as a reliable reference for epileptic seizure prediction, as well as an important target for anti-epileptic drugs [3], so it is important to investigate the underlying neuronal mechanism.

Some fundamental issues, such as where IIDs and IDs arise and how the transition of IIDs to IDs occurs, are essential for characterizing the dynamic changes in the neuronal network underlying the transition. Both clinical and experimental evidence suggest that the neonatal hippocampus is vulnerable to developing IDs from IIDs [7, 8]. A study based on rat hippocampal slices using animals aged from postnatal day 1 to day 30 (P1 to P30) suggested that IDs can easily be elicited in hippocampal slices during the first three postnatal weeks [9]. Multiple-site recordings made from combined hippocampus-

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entorhinal cortex slices or isolated hippocampal slices from juvenile rats have revealed that IIDs and IDs originate and are maintained in the hippocampus, and IIDs arise from the CA3a subfield, while IDs arise from the CA3c subfield [4].

Taken together, these findings suggest that, compared with adults, the hippocampus of neonatal or juvenile rodents is more vulnerable to developing IDs from IIDs. However, the mechanism underlying this transition is still unclear. Studies based on extracellular recordings suggest that, in the developing rat hippocampus, the excitatory GABA effect mediated by GABA_A receptors is responsible for increased seizure susceptibility and leads to the sudden onset of ictal activity [9, 10]. It has also been suggested that a shift in the reversal potential of GABA_A-receptor-mediated Cl[−] current contributes to ictogenesis [11]. However, the contribution of GABA_A receptor activity to seizure onset has been challenged by reports that, during the transition from IIDs to IDs in the immature mouse hippocampus, the GABA_A-receptor-related hyperpolarizing responses are maintained [12]. Although consensus has still not been reached, these results have argued for a close relationship between GABA_A receptors and the transition from IIDs to IDs. Hence, more investigations are needed to study the role that GABA_A receptor-mediated activity plays in the transition process in juvenile rodents.

Therefore, in this study we determined the dynamic changes in the transition process and explored how GABA_A receptor-mediated action affects the onset of IDs. This will enrich our knowledge on ictogenesis and provide insights into epilepsy treatment.

Materials and Methods

Hippocampal Slice Preparation

Fourteen juvenile male C57BL/6J mice aged 2 weeks–3 weeks were purchased from Shanghai Institutes for Biological Sciences. All animal handling and experiments were approved by and performed in accordance with the regulations of the Ethics Committee, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. All efforts were made to reduce the number of animals used and to minimize their suffering.

After anesthesia by isoflurane inhalation, the mice were killed by cervical dislocation. The brain was removed rapidly (within 2 min) and placed in ice-cold artificial cerebrospinal fluid (ACSF) consisting of (in mmol/L) NaCl 124.0, KCl 3.5, CaCl₂ 2.5, NaH₂PO₄ 1.2, MgCl₂·6H₂O 1.3, NaHCO₃ 25.0, and glucose 10.0. Transverse hippocampal slices were cut at 400 μm on a vibratome (Series 1000, Tissue Sectioning System, The Vibratome Company, St. Louis, MO) and kept in ACSF at room temperature (25°C–

28°C) for at least 2 h before use. No more than one slice per animal was used under each experimental condition. The pH of all solutions was adjusted to 7.4, and they were continuously gassed with a mixture of 95% O₂ and 5% CO₂.

Micro-Electrode Array (MEA) Recordings

Each MEA (MEA60, Multi Channel Systems GmbH, Reutlingen, Germany) consisted of 60 recording electrodes, 30 μm in diameter with 200-μm tip-to-tip inter-electrode spacing (horizontal and vertical). The 60 electrodes were arranged in an 8 × 8 matrix, leaving 4 corners void. Signals were amplified by a 60-channel amplifier (single-ended, bandwidth 1 kHz–3.4 kHz, amplification 1200×, input impedance > 10¹⁰ Ω, output impedance 330 Ω). An Ag/AgCl wire immersed in the bath served as the reference electrode. A hippocampal slice was transferred to the recording chamber, and the relative position of the slice on the MEA was ascertained appropriately by observation under a microscope (Olympus, Tokyo, Japan). A nylon mesh was used to prevent the slice from moving. To evoke epileptiform discharges, slices were perfused with Mg²⁺-free ACSF in which MgCl₂·6H₂O was omitted from the normal ACSF without substitution. Slices were maintained at 35°C–36°C with a temperature control unit (Thermostat HC-X, Multi Channel Systems GmbH) and the perfusion rate (2 mL/min) was controlled by a peristaltic pump (Ismatec SA, Glattburg-Zrich, Switzerland). Data were sampled at 20 kHz and stored in a personal computer for offline analysis [13–16].

Pharmacology

Muscimol (MUS) and bicuculline (BIC) from Sigma-Aldrich (St. Louis, MO) were dissolved in Mg²⁺-free ACSF. The bath concentrations of MUS were 3 μmol/L and 10 μmol/L, and that of BIC was 10 μmol/L.

Data Analysis

MC_Rack 4.1.1 (Multi Channel Systems GmbH) was used to record and display data. Offline analysis was performed using programs written with MatLab 7.10.0 (MathWorks, Natick, MA). To obtain local field potentials (LFPs), raw data were filtered with a band-pass filter (1 Hz–100 Hz). To detect the LFPs of the epileptiform discharges, a threshold was set at four standard deviations above the baseline, which did not contain epileptiform discharges during the first 10 min of perfusion with Mg²⁺-free ACSF. To obtain the parameters of epileptiform discharges (frequency, duration, initiation site, and propagation speed), the data from all electrodes along the pyramidal

cell layer were included. The frequency of epileptiform discharges was calculated based on the burst count in the whole process of each stage of discharge. The duration of each epileptiform discharge event was calculated based on the first and last time points that exceeded the threshold. As suggested in previous studies [4, 14, 17], the channel in which the earliest negative peak appeared was defined as the initiation site, and the propagation of the epileptiform discharges was judged by comparing the occurrence time of the first negative peak of LFPs, and the velocity of propagation was measured based on the tip-to-tip distances between adjacent electrodes and the relative latencies of the signals along the pyramidal cell layer.

Statistics

The data collected from 14 slices were analyzed. Measurements are expressed as mean $\pm$ SEM and error bars indicate SEM. The statistical significance of differences was assessed using Student's *t*-test or one-way ANOVA with a significance level set at $P < 0.05$.

Results

Epileptiform Discharges Induced by Mg^{2+} -Free ACSF in Hippocampal Slices

Recurrent Seizure-Like Events (SLEs)

After a hippocampal slice was exposed to Mg^{2+} -free ACSF for 10 min–20 min (13.9 ± 4.0 min, $n = 14$ slices), spontaneous and synchronous epileptiform discharges were recorded across the slice. Fig. 1A shows an example of a hippocampal slice mounted on an MEA. SLEs were recorded in all cornu ammonis (CA) and dentate gyrus (DG) regions (Fig. 1B–C), and occurred recurrently, with duration of 138.0 ± 32.28 s and an inter-SLE interval of 332.2 ± 112.0 s ($n = 14$ slices). Consistent with previous studies [11, 12], a typical SLE was classified into one of three states: inter-ictal-like, pre-ictal-like, and ictal-like (Fig. 1C). Brief (80 ms–200 ms) and sharp, the discharges during the interictal-like state were analogous to the interictal (between seizures) spikes in the electroencephalogram (EEG) [18] and were termed IIDs. The discharges in the ictal-like state lasted longer than 5 s and were analogous to the ictal pattern in the EEG when patients suffer seizures [19] and were termed IDs. Discharges during the pre-ictal-like state, which were much longer than IIDs (400 ms–600 ms) but shorter than IDs and occurred prior to IDs, were termed pre-ictal epileptiform discharges (PIDs) [11].

Transition from IIDs to IDs

Usually, there was a gradual process of change prior to the onset of an ID that included several recurrent IIDs and PIDs (Fig. 1). The duration of IIDs was 50 ms–200 ms (125.2 ± 50.1 ms, $n = 14$ slices). After 4–8 (5.7 ± 1.4) recurrent IIDs at inter-IID intervals of 9 s–17 s (13.1 ± 3.2 s), the discharges evolved into PIDs, which had longer durations than IIDs, ranging from 475 ms to 631 ms (553.6 ± 78.0 ms) with recurrent oscillations (Fig. 2A). There were usually 0–3 (1.3 ± 0.9) PIDs prior to the onset of an ID, with inter-PID intervals of 0.8 s–4 s (2.4 ± 1.6 s). The inter-PID intervals were much shorter than inter-IID intervals (Fig. 2A). The start of an ID was identified as a transition to a much longer epileptiform discharge (73.0 ± 32.8 s).

Generally, IIDs contained single spikes, while PIDs and IDs more often showed rhythmic oscillations. During the pre-ictal-like state, the rhythmic frequencies of oscillation were calculated from the total number of spikes over the entire duration of each PID. In the ictal-like state, the rhythmic frequencies were calculated from the number of spikes during the first 10 s after the ID onset [20]. There were no significant differences between the rhythmic frequency of PIDs (12.5 ± 2.4 Hz) and that of IDs (9.2 ± 1.8 Hz; Fig. 2B).

These findings suggested that, rather than an abrupt event, the onset of ID develops through a transitional process that contains several IIDs and PIDs. The IDs were preceded by an increase in discharge duration and a decrease in inter-discharge interval, and then transitioned into discharges at a high rhythmic frequency.

Initiation and Propagation of IIDs, PIDs, and IDs

The initiation site and spatial propagation of the three kinds of epileptiform discharge were further analyzed. By comparing the occurrence time of the first negative peak of LFPs in the pyramidal cell layer, we found that the initiation sites of IIDs, PIDs, and IDs were not necessarily identical in different slices. In most slices (13 out of 14), the discharges initiated in CA3 (CA3a in 5 slices and CA3b in 8 slices), then bi-directionally spread to CA1 (anterograde) and CA3c (retrograde) (Fig. 3A–C). But there was also one exceptional slice, in which the IIDs initiated in CA3c and the initiation site of PIDs was in CA3a (data not shown). The propagation speeds of the IIDs in the two directions (anterograde and retrograde) were 202.7 ± 38.5 mm/s and 125.3 ± 28.6 mm/s, while those of the PIDs were 192.5 ± 76.8 mm/s and 136.3 ± 25.8 mm/s, and those of IDs were 213.9 ± 70.4 mm/s and 139.0 ± 37.1 mm/s, respectively ($n = 13$). The propagation speeds of IIDs and IDs in the

Fig. 1 Epileptiform discharges in a juvenile mouse hippocampal slice. **A** Photograph of an acute hippocampal slice mounted on an MEA; black dots indicate the electrodes and the channels are labeled by numbers. **B** Typical recordings of epileptiform discharges from the slice shown in **A**. The data windows show recordings from the electrodes identified by numbers in the top right corners. **C** Representative recordings of epileptiform discharges in the pyramidal cell layer of the CA3a region (channel 31). The discharges were characterized by recurrent SLEs. A 180-s recording is plotted in the middle trace with the time scale expanded to show a typical SLE. Typical IID, PID, and ID are shown in the lower traces.

anterograde direction were significantly faster than those in the retrograde direction. However, there were no significant differences among the propagation speed of the three kinds of epileptiform discharge in the same direction.

Effects of GABA_A Receptor Activity on Recurrent SLEs

It has become increasingly clear that GABA_A receptor activity is involved in the generation of epileptiform discharges in juvenile rodents [4, 9, 20], so we further

Fig. 2 Characteristics of IIDs, PIDs, and IDs. **A** Durations and the inter-discharge intervals of IIDs and PIDs. The durations were longer and the intervals were shorter in PIDs than in IIDs (* $P < 0.05$, paired

t -test, $n = 14$). **B** Rhythmic frequencies of PIDs and IDs. There was no significant difference ($P > 0.05$, paired t -test, $n = 14$).

Fig. 3 **A–C** Initiation and propagation of the IIDs (**A**), PIDs (**B**), and IDs (**C**) in a representative hippocampal slice (that shown in Fig. 1A). These discharges all initiated in CA3a and propagated bi-directionally to CA1 (anterograde) and CA3c (retrograde). **D** Propagation speeds of the epileptiform discharges in interictal-like, pre-ictal-like, and ictal-

like states. There were no significant differences among the propagation speeds of the three kinds of discharge in the same direction ($P > 0.05$, paired t -test, $n = 13$). The anterograde propagation speed of the IIDs and IDs were significantly faster than their retrograde speeds (* $P < 0.05$, paired t -test, $n = 13$).

investigated the contribution of GABA_A receptor-mediated neurotransmission to SLEs.

Effects of 3 $\mu\text{mol/L}$ Muscimol on Recurrent SLEs

After the epileptiform discharges were stable for at least 20 min, the GABA_A receptor agonist MUS was applied (Fig. 4). When GABA_A receptors were partially activated by 3 $\mu\text{mol/L}$ MUS, PIDs and IDs disappeared, while IIDs persisted. The same phenomenon was found in three other

slices. These data showed that GABA_A receptor-mediated activity suppresses the transition from IIDs to IDs.

The application of MUS changed the temporal and spatial patterns of IIDs (Fig. 5). In the control condition and that with 3 $\mu\text{mol/L}$ MUS, the IID durations were 111.48 ± 6.87 ms and 168.72 ± 42.13 ms, and the inter-IID intervals were 9.15 ± 1.0 s and 2.71 ± 0.47 s, respectively. There was no significant difference in duration between the IIDs under the two conditions, while the intervals decreased significantly under the MUS condition.

Fig. 4 Effects of 3 $\mu\text{mol/L}$ muscimol (MUS) on epileptiform discharges. Upper trace: representative recording from the pyramidal cell layer of CA3a during perfusion with Mg^{2+} -free-ACSF (control) and with the application of 3 $\mu\text{mol/L}$ MUS. Middle and lower traces: discharges under the control condition and during MUS application at expanded time scales. The application of 3 $\mu\text{mol/L}$ MUS abolished the PIDs and IDs, while the IIDs persisted.

Fig. 5 Effects of 3 $\mu\text{mol/L}$ muscimol (MUS) on the IIDs. **A** Effect of MUS on the durations and inter-discharge intervals of IIDs. The duration was not influenced, while the interval decreased significantly ($P < 0.05$, paired t -test, $n = 4$). **B** Initiation of IIDs during control and with the application of 3 $\mu\text{mol/L}$ MUS. The IID initiation area shifted from CA3a to CA3b after MUS was added.

On the other hand, the IID initiation areas shifted from CA3a to CA3b in 3 slices after perfusion with 3 $\mu\text{mol/L}$ MUS (Fig. 5B), and in the other slice in which the IIDs initiated in CA3b, no change in initiation area occurred. The shift of initiation site of IIDs was possibly because the original IID pacemaker was suppressed and another new pacemaker emerged during 3 $\mu\text{mol/L}$ MUS application.

Effects of 10 $\mu\text{mol/L}$ Muscimol on Recurrent SLEs

MUS was also applied at a concentration of 10 $\mu\text{mol/L}$ to enhance the GABA_A receptor-mediated activity. This abolished all the epileptiform discharges in 8 min–20 min (13.9 ± 4.0 min, $n = 5$ slices; Fig. 6). These results suggested that GABA_A receptor activity has an inhibitory effect on the epileptiform discharges in juvenile mouse hippocampal slices in a dose-dependent manner.

Effects of Bicuculline on Recurrent SLEs

The GABA_A receptor antagonist BIC (10 $\mu\text{mol/L}$) was also used to confirm the effects of GABA_A receptor activity on SLEs. When BIC was added to the Mg^{2+} -free ACSF, the IIDs and PIDs were completely abolished, while IDs occurred more frequently (Fig. 7). The durations of IDs were significantly decreased (60.26 ± 25.10 s in control and 7.25 ± 1.21 s in BIC; Fig. 8A), as were the inter-ID intervals (436.23 ± 86.79 s in control and 35.15 ± 13.73 s in BIC; Fig. 8A). These results suggested that the absence of GABA_A receptor activity hastens the transition and results in the easy onset of IDs.

Despite these changes, the initiation sites and propagation directions of the IDs were unaffected (Fig. 8B). The propagation speeds of IDs in the anterograde and retrograde directions in controls were 227.61 ± 68.87 mm/s and 130.31 ± 47.55 mm/s ($n = 5$ slices), and those with BIC application were 169.01 ± 14.33 mm/s and 141.37 ± 64.43 mm/s, respectively, and the changes were not significant (Fig. 8C).

Fig. 6 Effects of 10 $\mu\text{mol/L}$ muscimol (MUS) on the epileptiform discharges recorded from the pyramidal cell layer of CA3a during perfusion with Mg^{2+} -free-ACSF. All the discharges disappeared after the slice was perfused with MUS for 10 min.

Discussion

Our results showed that, in juvenile mouse hippocampal slices exposed to Mg^{2+} -free ACSF, the transition from IIDs to IDs was preceded by increased discharge durations and decreased inter-discharge intervals (Figs 1 and 2). Activating the GABA_A-receptor-mediated activity with MUS blocked the transition from IIDs to IDs (3 $\mu\text{mol/L}$, Fig. 4), while blocking the GABA_A-receptor-mediated activity increased the occurrence of IDs (Fig. 7). These results suggest that a transition process occurs prior to the onset of IDs, and GABA_A-receptor-mediated activity suppresses the transition from IIDs to IDs.

There is a Transition Process Prior to Seizure Onset

Until recently, the onset of a seizure was believed to be an abrupt phenomenon, occurring without warning [3]. However, evidence from clinical and physiological studies suggested that there was a transition process before the onset of IDs. And non-specific ‘prodromal’ symptoms had been reported in patients with temporal lobe epilepsy, including mood changes, irritability, sleep problems, nausea, or headache [21]. By assessing the dynamic changes of the metabolic index at different moments prior to seizure onset, a decrease in metabolic rate and neuronal activity in the brain has been found to occur prior to the onset of temporal lobe seizures [22]. The data from intracranial EEG recording from patients with partial epilepsy have shown that the transition between the inter-ictal and ictal states does not occur abruptly and that a third state exists (mean duration ~ 7 min), the pre-ictal state, which is a state of network activity transitional between the inter-ictal and ictal states [23]. All these results suggest that there is a transition process prior to the onset of IDs in the epileptic brain. This transition period has been recognized as a reliable reference for epileptic seizure prediction, as well as an important target for antiepileptic drugs [3, 5].

In parallel with these clinical findings, a transitional process from IIDs to IDs has also been found in physiological experiments [4, 11, 24]. In the present study, we established a model in the juvenile mouse hippocampal slice using Mg^{2+} -free ACSF perfusion. The onset of seizures in this model was characterized by recurrent SLEs, and the transition process from IIDs to ID was found in each SLE. In agreement with previous studies [4, 11, 25], these changes in our experiments reflect the gradual build-up of a network state that can be referred to as ictogenesis prior to the onset of IDs.

Consistent with previous reports [14, 26], most of the epileptiform discharges were initiated in the CA3a/b regions and spread bi-directionally to CA1 (anterograde)

Fig. 7 Effects of 10 $\mu\text{mol/L}$ bicuculline on the epileptiform discharges. Upper trace: representative recording in the pyramidal cell layer of CA3a during perfusion with Mg^{2+} -free-ACSF (control) and with the application of 10 $\mu\text{mol/L}$ BIC. Middle and lower traces: discharges plotted at expanded time scales. BIC abolished the IIDs and PIDs, while IDs occurred at a higher frequency.

and CA3c (retrograde) in hippocampal slices (Fig. 3). Increased self and cross synaptic connectivity in the CA3 regions may account for the ability of pyramidal cells to generate epileptiform activity [26, 27]. Furthermore, the apical dendrites of CA1 pyramidal cells are innervated predominantly by neurons in the CA3a region, and CA3 pyramidal cells also send axon collaterals back to the hilar region [27]. These structures support the anterograde and retrograde propagation. Differences in the biophysical properties and generation mechanisms of the epileptiform discharges may be attributable to the differences in propagation characteristics [18]. In most of the slices investigated in our experiments (13 out of 14), the initiation area and propagation speed did not change significantly in the transition, indicating that neither of these is the main factor determining the transition process. This result is inconsistent with the results reported by Dzhalal *et al.*, who found a shift in the IID initiation area

from CA3 a/b to CA3c as well as that ictal-like activity originating in CA3c was followed by spread to CA1, from recordings of epileptiform discharges induced by increasing the extracellular concentration of K^+ in hippocampal-entorhinal cortex slices from male Wistar rats at P10 to P20 [4]. So, the differences between our results and previous reports are most likely due to the species used, thickness of the slice, and other technical issues that affect *in vitro* experiments [4, 28].

Effects of GABA_A Receptor-Mediated Actions on the Onset of IDs

GABA is one of the inhibitory transmitters in the mammalian brain [29]. GABA_A receptor-mediated activity is understood to be involved in the generation of epileptiform discharges in juvenile rodents [4, 9, 20]. Some researchers agree that excitatory action of GABA *via*

Fig. 8 Effects of bicuculline on IDs. **A** BIC at 10 $\mu\text{mol/L}$ decreased the durations and intervals of IDs ($P < 0.05$, paired t -test, $n = 5$). **B** The initiation site of an ID during control and with BIC in a representative slice. As in the control condition, the IDs were generated in the pyramidal cell layer of CA3a during the application of BIC. **C** There was no significant effect BIC on the propagation speed of IDs ($P > 0.05$, paired t -test, $n = 5$).

GABA_A receptors is responsible for the increased seizure susceptibility in the developing rat hippocampus [9, 10, 30, 31]. Staley *et al.* reported that the excitatory action of GABA depends on a high intracellular Cl⁻ concentration and a depolarized Cl⁻ equilibrium potential, leading to a depolarization response mediated by GABA_A receptors [32]. Despite these findings, an alternative view is that in juvenile animals the action of GABA mediated by GABA_A receptors is inhibitory [8]. Studies based on the immature mouse hippocampus have shown that the inhibitory synaptic current is maintained throughout the entire transition period from inter-ictal to ictal discharges [12]. Species differences and the ages of experimental animals used may influence the argument.

Our data showed that GABA_A receptor-mediated activity restrains the transition from IIDs to IDs, and the IDs are generated more easily in the absence of such activity. Therefore, our data support the hypothesis that GABA_A receptors mediate an inhibitory action on the SLEs induced

by Mg²⁺-free ACSF in the juvenile mouse hippocampal slice. The evidence from whole-cell patch-clamp recordings made in the hippocampus of male black C57 mice at P6–P12 shows that GABA_A-mediated inhibitory postsynaptic currents (IPSCs) predominate in the inter-ictal and the early pre-ictal states, and these IPSCs then decrease to zero by the onset of IDs [20]. So, our results are in agreement with the speculation that the onset of an ID is heralded by exhaustion of the presynaptic release of GABA.

In conclusion, our results indicate that dynamic changes in the hippocampal network precede the onset of IDs, and it seems that GABA_A receptor-mediated actions suppress the transition from IIDs to IDs in the juvenile mouse hippocampus. Based on the results of the pharmacological experiments, we speculate that the mechanisms underlying the onset of IDs are related to the attenuation of GABA_A receptor-mediated inhibitory activity.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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