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RESEARCH ARTICLE

Simultaneous calcium recordings of hippocampal CA1 and primary motor cortex M1 and their relations to behavioral activities in freely moving epileptic mice

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Abstract

Epilepsy is a common neurological disorder characterized by recurrent epileptic seizures. The cause of most cases of epilepsy is unknown. Although changes of calcium events in a single brain region during seizures have been reported before, there have been few studies on relations between calcium events of two different brain regions and epileptic behaviors in freely moving mice. To analyze calcium events simultaneously recorded in hippocampal CA1 (CA1) and primary motor cortex M1 (M1), and to explore their relations to various epileptic behaviors in freely moving epileptic models. Epileptic models were induced by Kainic acid (KA), a direct agonist of glutamatergic receptor, on adult male C57/BL6J mice. Calcium events of neurons and glia in CA1 and M1 labeled by a calcium indicator dye were recorded simultaneously with a multi-channel fiber photometry system. Three typical types of calcium events associated with KA-induced seizures were observed, including calcium baseline-rising, cortical spreading depression (CSD) and calcium flashing with a steady rate. Our results showed that the calcium baseline-rising occurred in CA1 was synchronized with that in M1, but the CSD waves were not. However, synchronization of calcium flashing in the two areas was uncertain, because it was only detected in CA1. We also observed that different calcium events happened with different epileptic behaviors. Baseline-rising events were accompanied by clonus of forelimbs or trembling, CSD waves were closely related to head movements (15 out of 18, 6 mice). Calcium flashing occurred definitely with drastic convulsive motor seizures (CMS, 6 mice). The results prove that the synchronization of calcium event exists in CA1 and M1, and different calcium events are related with different seizure behaviors. Our results suggest that calcium events involve in the synchronization of neural network and behaviors in epilepsy.

Keywords Epilepsy · Behavior · Hippocampal CA1 · Primary motor cortex M1 · Multi-channel fiber photometry

Abbreviations

KA	Kainic acid
CA1	Hippocampal CA1
M1	Primary motor cortex M1
CSD	Cortical spreading depression
EEG	Electroencephalography

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Xi Dong and Xin Zhang contributed equally to this work.

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CMS Convulsive motor seizure
SE Status epileptics
STFT Short-time Fourier transform
FFT Fast Fourier transform

Introduction

Epilepsy is characterized by epileptic seizures, transient symptoms of abnormal excessive or synchronous neuronal activity in the brain (Fisher et al. 2005). Its pathogenesis is related to the unbalance of neuronal excitation and inhibition at the cellular level or neuronal network level (Kaila et al. 2014). Calcium homeostatic mechanism is considered to be important to the development and maintenance of epilepsy (Delorenzo et al. 2005). Abnormal intracellular calcium concentration is crucial to the origination or propagation of epileptic seizures (Delorenzo et al. 2005; Scharfman 2007). Since the calcium ion (Ca^{2+}) is a major second messenger affecting a variety of neurological diseases including epilepsy (Ugawa 2013; Xu and Tang 2018), it is reasonable to assume that this underlying Ca^{2+} change plays a vital role in epilepsy. Thus, to investigate the change of calcium signals in the brain is important in the prevention and treatment of epilepsy.

Epilepsy induced by Kainic acid (KA), a direct agonist of glutamatergic receptor, is very similar to temporal lobe epilepsy involved in many areas of brain (Kim 2015). Altered calcium events had been observed during KA-induced seizures in a single brain area such as hippocampal CA1, CA3, dentate gyrus and entorhinal cortex (Zhang et al. 2019). M1 is also crucial for diagnosis and therapy of epilepsy, according to a recent critical review focus on the relation between the primary motor cortex and epilepsy (Ostergard and Miller 2019). However, little is known about the relation between calcium events in CA1 and M1, because no research has been performed to the simultaneous recording and analyzing of calcium events from two or more brain regions in an epileptic model, especially in a freely moving epileptic animal model. Meanwhile, calcium events are assumed to have some relation to seizure behaviors (Berdyyeva et al. 2016). But so far, there are few reports about the relation between characteristic calcium events and specific epileptic behaviors.

Multi-channel fiber photometry is an efficient calcium signal acquisition system with superior sensitivity (Gunaydin et al. 2014) and high signal–noise ratio (Kim et al. 2016). The surgical process of multi-channel fiber photometry is safer than that of micro-endoscopic calcium imaging device (Berdyyeva et al. 2016). Therefore, this technique has its advantages in simultaneous recording of calcium events in multiple brain regions, so it has been widely used for functional studies of neural circuits in freely moving animals (Allen et al. 2017; Guo et al. 2015).

In this study, we used freely moving epileptic animal model induced by KA to analyze the possible synchronism of calcium events simultaneously recorded in CA1 and M1 by multi-channel fiber photometry and the connection of characteristic calcium events with specific seizure behaviors.

Materials and methods

Animals and treatments

Adult male C57/BL6J mice ($n = 11$) used for experiments were aged 8–12 weeks, weighing 20–25 g and obtained from the institute of zoology, Chinese academy of sciences. All animals were group housed 5–6 per cage over a corn-cob bedding, maintained at 23 ± 2 °C and $50 \pm 10\%$ humidity under a 12 h light/dark cycle (lights on at 7:00 am) and fed ad libitum with tap water and food. Mice in the same cage were randomly allocated to each experimental group by sampling across different cages. Efforts to minimize the number of animals and reduce pain were undertaken in the experimental design. To avoid the interference of hormones, only male mice were used in our study. All Procedures were approved by the Animal Care and Use Committee of Tianjin Medical University, in accordance with National Institutes of Health guidelines.

Drug administration

The KA (K0250, Sigma, USA) was formulated in 0.9% sodium chloride and was injected intraperitoneally into mice in proportion to their body weight (10 mg/kg) before each recording session. Fluorescent dye Cal-520 AM is a reliable Ca^{2+} indicator with a sufficient sensitivity and a better signal–noise ratio) in both anesthetized and awake conditions, both neurons and astrocytes can be labeled by Ca-520 AM (Li et al. 2017; Tada et al. 2014). Therefore, Cal-520 AM (AAT Bioquest, USA) was used for imaging of cellular calcium events. Isoflurane (R510-22, RWD Life Science, China) was used to induce or maintain anesthesia in mice.

Fiber photometry system

Multi-channel fiber photometry system (ThinkerTech, Nanjing; Fig. 1a) was used for cellular calcium event recordings. Real-time fluorescence intensity of each optical fiber in the multi-mode optical fiber bundles was collected using CMOS array. Excitation light at 480 nm was transmitted to CA1 and M1 through end-separated multi-mode fiber bundles after each optical fiber was, respectively, inserted into (Guo et al. 2015). Then averaged fluorescence intensity of

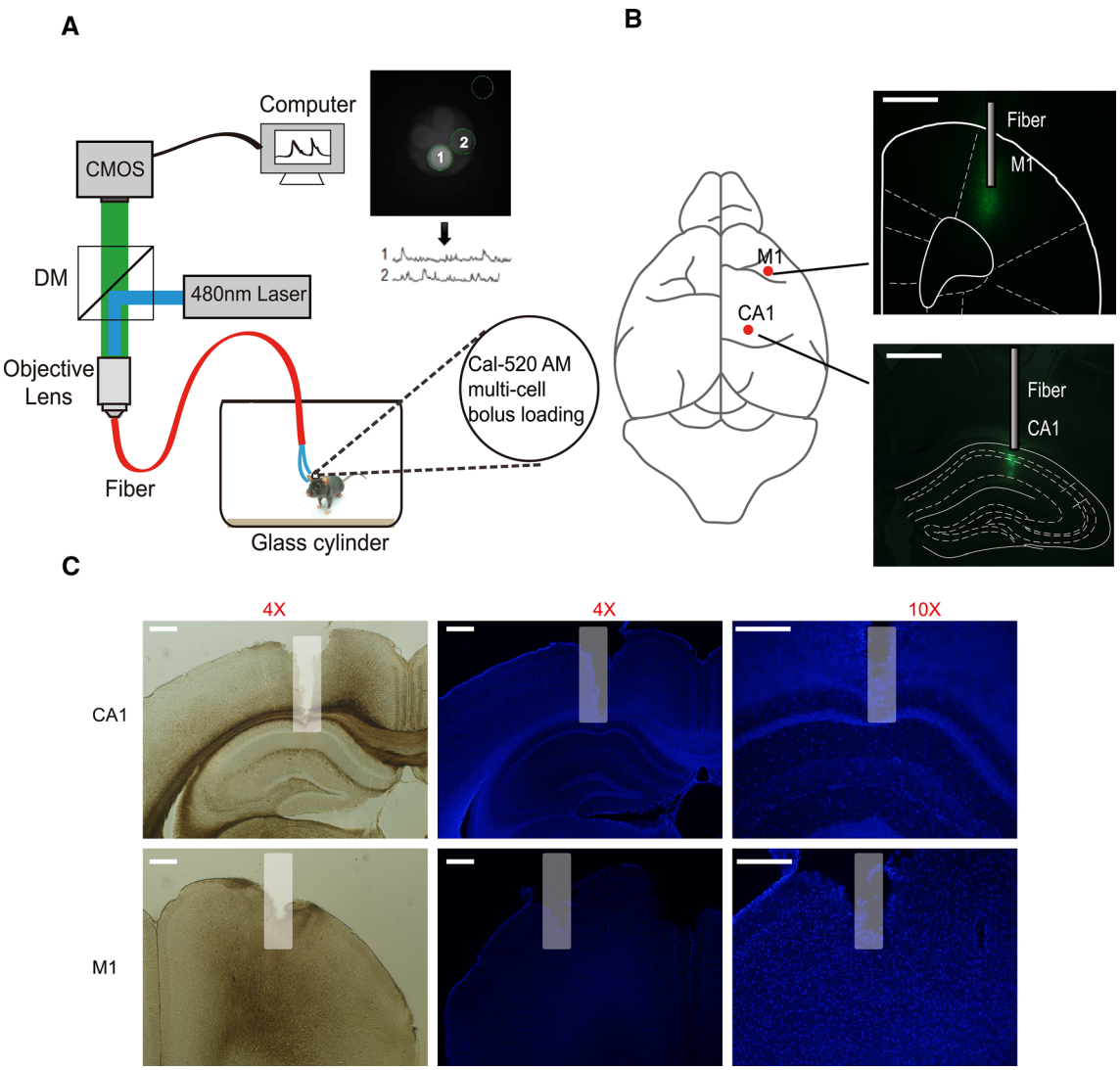

Fig. 1 Histology and fluorescence imaging of CA1 and M1. **a** Schematic diagram of fiber photometry system used for calcium signals recording. **b** Schematic diagram showing the exact position where optical fibers were implanted into hippocampal CA1 and M1 separately on the surface of the cerebrum. Green fluorescence represents brain regions stained with calcium dye Cal-520 AM. The bar in the upper left corner represents an actual distance of 1.0 mm. **c** Histology and fluorescence imaging of CA1 and M1. Three subgraphs above are accurate localization of histology and fluorescence imaging of CA1 with both × 4 and × 10 objective, separately. Subgraphs below illustrating histology and fluorescence imaging of M1. Actual distance of bars here is 0.25 mm. Gray bars show fiber tracks of each brain region

each brain region was detected and converted into digital calcium signals.

Animal surgery and multicellular loading of dye Cal-520 AM

Mice were narcotized in a plastic transparent chamber with 2.5% isoflurane for 2 min and head-fixed into a stereotaxic frame on a heating pad for maintaining physiological temperature, 0.8–1.5% isoflurane was used to maintain anesthesia during surgery processes. Ophthalmic ointment was used for protecting animals' eyes from drying. Two 1 mm diameter holes on the surface of skull were drilled for Cal-520 AM injection of hippocampal CA1 (anteroposterior: 2.15 mm, mediolateral: ± 1.45 mm, dorsoventral: 1.25 mm) and primary motor cortex M1 (anteroposterior: 2.00 mm, mediolateral: ± 1.80 mm, dorsoventral: 0.60 mm) (Fig. 1b), respectively. Animal surgery and dye injection (~ 100 nl) were executed carefully and scrupulously following the procedure guideline reported before (Adelsberger et al. 2014; Guo et al. 2015). Neuronal and glial cellular loading of the calcium-sensitive dye Cal-520 AM (Li et al. 2017) in CA1 and M1 lasted 30 min. Then, tips of two optic fibers were placed into two regions gently through each hole opened for dye injection before. Dental cement and dental water were used to harden fibers onto the skull. Finally, calcium signals

in two brain regions and behavior monitoring videos were acquired simultaneously.

In vivo Ca^{2+} recording

Recording of calcium signals under KA administration was 1.5-h after surgery. Interval was vacated for animals' recovering in their original cages. Mice were placed into a transparent round glass cylinder with a diameter of 35 cm in which they could move freely and their performance would be well monitored from the observer. Then, the mice had 30 min to adapt to the environment, after which calcium events of CA1 and M1 were recorded simultaneously using the multi-channel fiber photometry system, corresponding behavioral activities after KA-injection were also supervised.

Histology and fluorescence imaging

To testify the Cal-520 AM staining and confirm the relative position of two optical fibers, all recorded animals were perfused transcardially with 4% paraformaldehyde in phosphate-buffered saline (PBS) after recordings. Treatment of brain samples after perfusion followed procedures reported before (Zhang et al. 2017b). Brain samples were cut into 30-μm-thick slices before nuclei were stained using 40, 6-diamidino-2-phenylindole. Images were captured using a fluorescence microscope (BX51, Olympus) and 4×(or 10×) objective (Fig. 1c).

Ca^{2+} signal processing

Simulative Ca^{2+} transients were obtained at a sampling rate of 50 Hz. Ca^{2+} transients were calculated as $\Delta F/F = (f - f_{\text{baseline}})/f_{\text{baseline}}$ to represent relative fluorescence changes without the interference of dye injection dose and baseline fluorescence intensity, where the f_{baseline} was the baseline level of fluorescence determined during the current recording period of the test (Guo et al. 2015). Pre-processing (including baseline correction, threshold-based event detection), event start time, event end time and peak amplitude search were carried out by Clampfit (version 9). Probability of the calcium baseline-rising (Fig. 2d), the coupling relationship of calcium events in CA1 and M1 (Fig. 2e), time interval of peak amplitude (Fig. 2f) and the time–frequency analysis of flashing (Fig. 4d, e) were all calculated with custom program based on MATLAB_2018a, a window with 10 points overlap were designed to achieve time–frequency analysis. Correlation analysis of different brain regions was blind to research observer of each experiment. To distinguish subtle fluorescence change, Ca^{2+} transients were normalized between 0 and 1 before amplitude comparison of different groups, where 0 represents the relevant minimum fluorescence change and 1 represents the maximum change (Fig. 4d, e).

Statistical analysis

GraphPad Prism 7.0 software was used for statistical analysis. Diagrams designs were achieved by Adobe Illustrator CS6 (64 bits). Background purification was achieved with Adobe Photoshop CS6 (Fig. 4b). Initially, all data were tested with the Kolmogorov Smirnoff test for normality and confirmed to be normally distributed except for noted. Parametric or nonparametric tests were used depending on the type of distribution observed. All data for repeated-measures two-way ANOVA were tested for sphericity in advance and multiplicity adjust P values were provided for more power. The Pearson's correlation coefficient of R was used to the cross-correlation of calcium baseline-rising in CA1 and M1 (Fig. 2e). Wilcoxon matched-pairs signed rank test was used to analyze the difference of event start time (Fig. 2b), event duration (Figs. 2c, 3c) and the average rising slope (Fig. 3b) between different calcium events in CA1 and M1. In addition, the duration of CSD were compared with the duration of build-up happened before it using the unpaired t test, which was corrected with Welch's test because of their unequal variances ($F_{12,13} = 3.772$; $n = 6$; $P = 0.0311$) (Fig. 3c), and all values are expressed as mean ± SD (standard deviation) based on the number of independent experiments.

Results

Results of histology and fluorescence imaging

Histology imaging showed that the accurate localization of brain regions where were operated stereotactically in CA1 and M1 (Fig. 1b); valid Cal-520AM staining of both CA1 and M1 was verified with fluorescence imaging (Fig. 1c). Only mice with correct operating sites and successful Cal-520 AM fluorescent coloration for both CA1 and M1 were included in our study (3 mice were excluded). Additionally, experimental subjects died for sharp seizures ($n = 2$) were excluded. Finally, a total of 6 (out of 11) mice were included in complete research.

Various epileptic behaviors accompanying with different real-time calcium events

Seizure behaviors were monitored and scored into five stages according to Racine scale (Racine 1972). For the importance of calcium events and complex coactions of multiple brain regions to seizures (Bui et al. 2015), we simultaneously recorded the calcium events in CA1 and

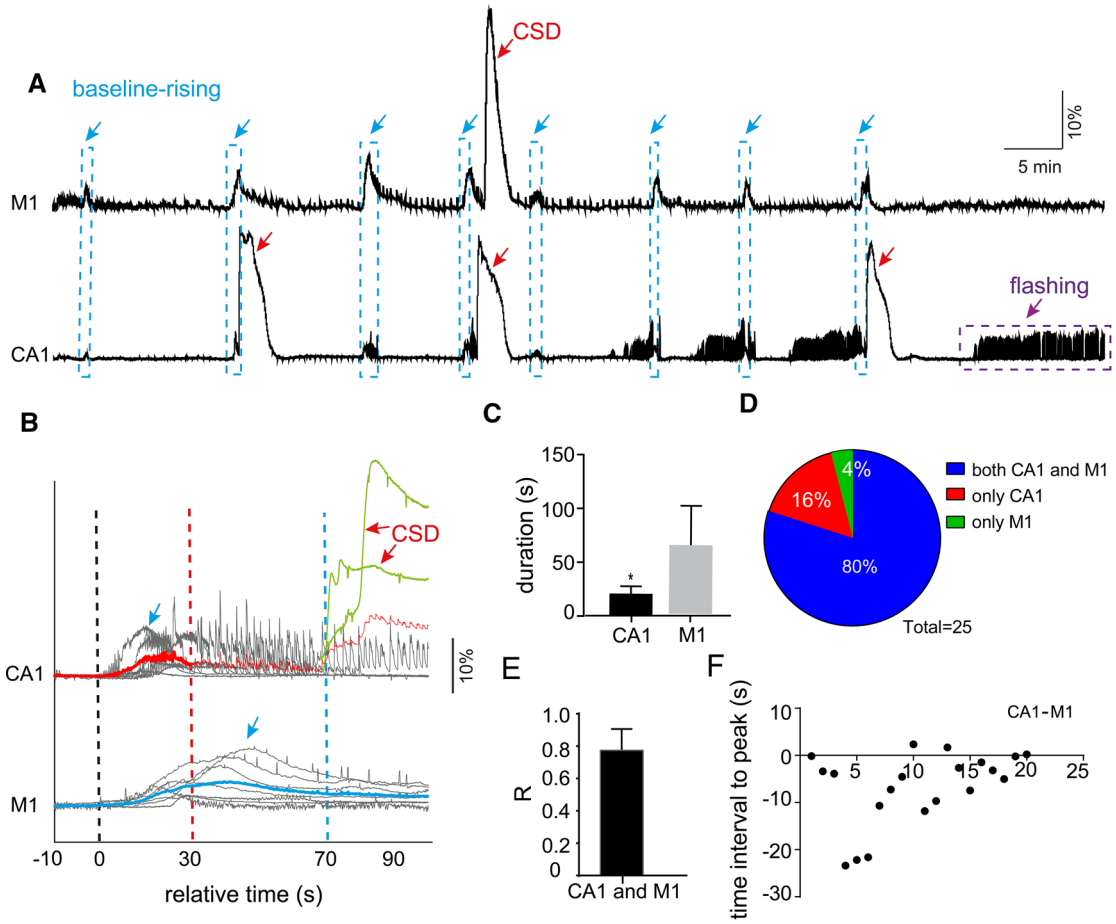

Fig. 2 High probability of simultaneous occurrence of significant baseline-rising events in both brain regions after KA administration. **a** Continuous calcium signals lasting for 1.5 h simultaneously recorded from CA1 and M1. These events of baseline-rising are marked by blue arrows. The CSD-like events are marked by red arrows and the flashing events are marked by the purple arrow. **b** Average (red and bold) calcium baseline-rising events occurred in CA1 and their corresponding average calcium signals (blue and bold) in M1. The timeline is intentionally started where baseline-rising in CA1 generated, which is marked as 0 (the black dotted line). The termination of baseline-rising events in different brain regions is marked by another two dotted line, CA1, red; M1, blue, $n=7$ trials from 6 mice. **c** The duration of typical baseline-rising events in CA1 and M1, these events immediately followed by CSD or calcium build-up were excluded from this analysis ($n=11$ trials). **d** Probability of calcium baseline-rising events happened simultaneously in two regions or in a single brain area. **e** The value of cross-correlation of the transient rising signals in CA1 and M1. **f** Time interval to peak amplitude compared CA1 and M1, results were obtained by subtracting the time to peak fluorescent values of CA1 from that of M1

M1 and explored the spatiotemporal relevance of them. Both neurons and astrocytes in CA1 and M1 were labeled by Cal-520 AM (Li et al. 2017). Interestingly, we observed three kinds of typical real-time calcium events on freely epileptic moving mice in our study, including the calcium baseline-rising occurred with lifted signals (Fig. 2a, blue arrow), the CSD-like event (Fig. 2a, red arrow) and the calcium flashing (Fig. 2, purple arrow) with steady and low event rate (Berdyyeva et al. 2016). In addition, our results showed that different epileptic behaviors would happen once calcium events mentioned above occurred in CA1 and M1.

Synchronization of baseline-rising events and their relations to seizure behaviors

Calcium baseline-rising was defined as an event with a transient rise in calcium fluorescence after KA administration in our study, sometimes it occurred before a cluster of calcium build-up with a relevant high-event rate or CSD-like events (Fig. 2a, b). To analyze the calcium kinetic and the possible synchronization of baseline-rising events in CA1 and M1, we separately calculated the event start time and the duration of them. No significant difference of event start time between these events in CA1 and M1 was observed ($P=0.3851$; Fig. 2b), but the duration of this kind of event in CA1 was

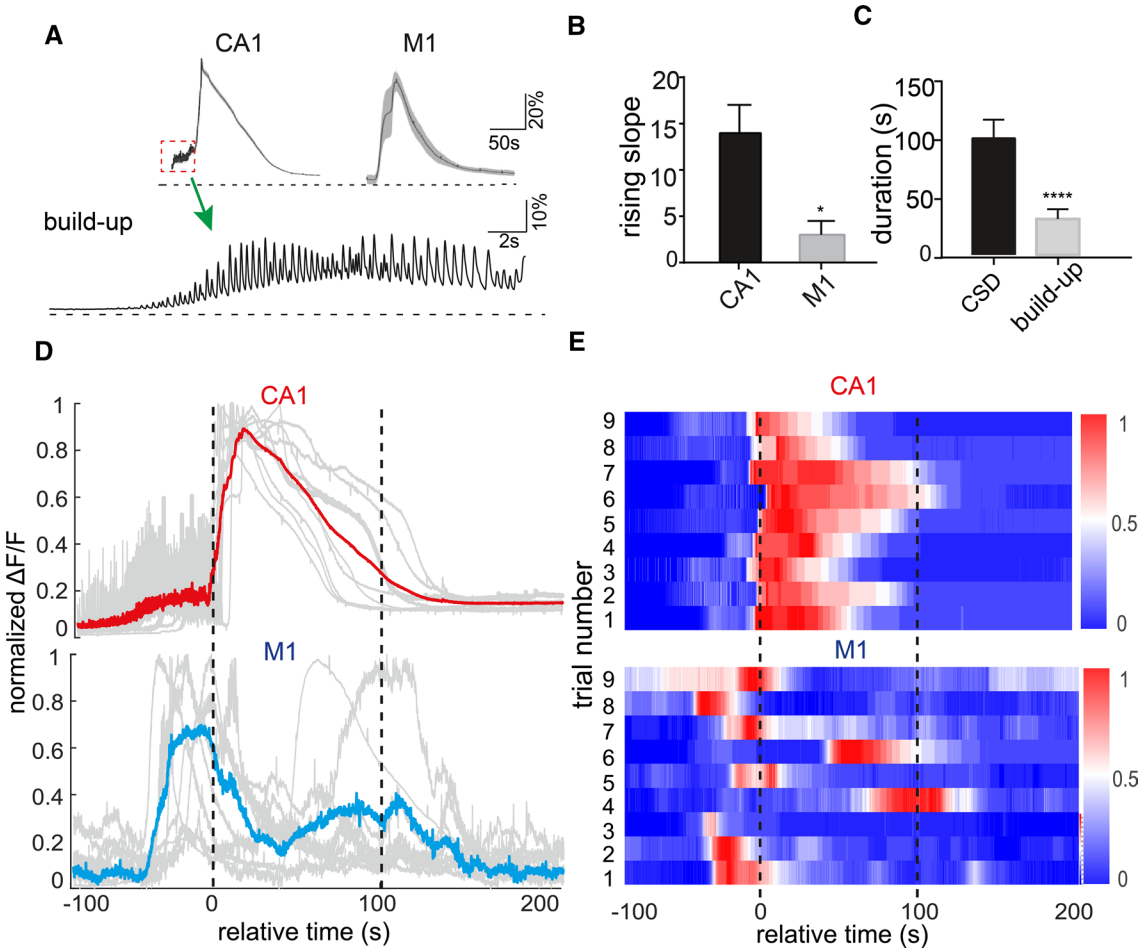

Fig. 3 Association between cortical spreading depression happened in CA1 and seizure-like behaviors induced by KA. **a** Mean CSD-like events observed in CA1 and M1 (upper), and the calcium build-up happened before CSD-like events in CA1(lower, labeled 'build-up'). **b** The average rising slope of CSD-like evens in different brain regions. **c** Duration comparison of CSD and corresponding calcium build-up in CA1. ($n=8$–13 trials from 6 mice, ***$P<0.0002$, ****$P<0.0001$ vs. the duration of CSD in CA1 separately, unless line segments were used.) **d** Calcium signals simultaneously recorded in CA1 and M1. The timeline is intentionally started where CSD generated, which is marked as 0 ($n=9$ trials from 6 mice). Averaged signals (bold) demonstrated the underlying relationship between seizure-like head movements observed and calcium activity change in both CA1 and M1. **e** Color-coded intensity of Ca^{2+} transients from different trials corresponding each signal presented in **d**, a long duration of CSD-like events was presented

significantly shorter than that in M1 ($n=8$; $P=0.0156$; Fig. 2c). Moreover, baseline-rising events in CA1 were closely related to CSD-like events, but baseline-rising events in M1 were not (Fig. 2a, b). Whereas our results showed that these calcium baseline-rising events in CA1 were largely correspond to those in M1 (Fig. 2d), specifically, once calcium baseline-rising events occurred in CA1, most calcium fluorescence in M1 would have a simultaneous transient rise (80%, $n=20$), but very few calcium baseline-rising events would appear alone in CA1 (16%, $n=4$) or M1 (4%, $n=1$). Meanwhile, the results of cross-correlation of baseline-rising events showed that the transient rise of calcium fluorescence in M1 was significantly associated with that in CA1 ($R=0.79$; Fig. 2e). Notably, the results showed that the time interval of baseline-rising events in CA1 to peak amplitude was significantly shorter than that in M1 ($P<0.001$; Fig. 2f).

Seizure behaviors like clonus of forelimbs and trembling were found to be accompanied with calcium baseline-rising in our study (stage 3; Online Resource 1). Specially, epileptic head movements ($n=10$; 6 mice; stage 2; Online Resource 2) or violent epileptic attacks with clonic seizures ($n=2$; 6 mice; stage 4; Online Resource 3) would happen once this kind of event were followed by calcium build-up or CSD-like events.

Synchronization of CSD-like events and their relations to specific head movement

The second kind of characteristic calcium event we observed both in CA1 and M1 was CSD-like event (Fig. 3a), which

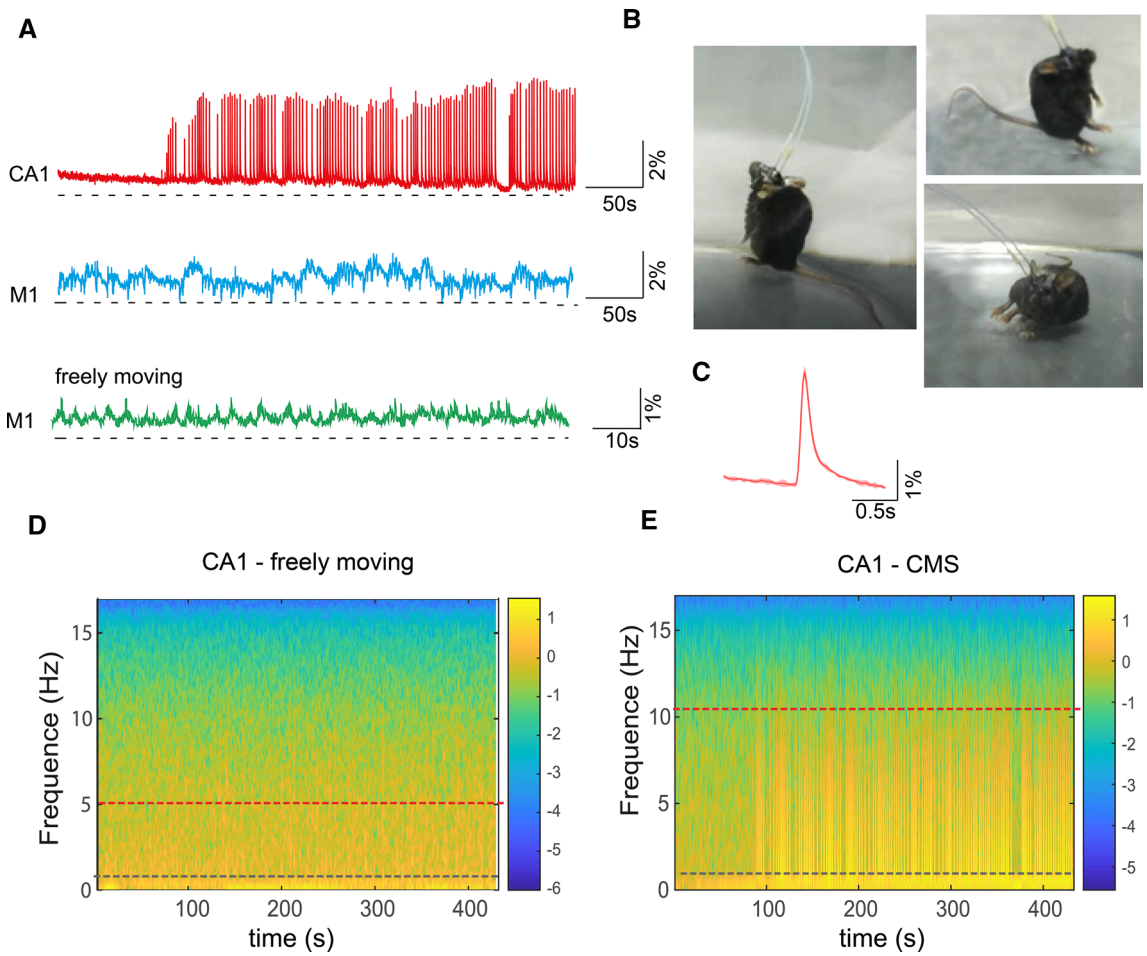

Fig. 4 Specific calcium flashing of CA1 during CMS identified using short-time Fourier transform. **a** Representative photometry recordings along with CMS conducted from CA1 after KA administration (upper) and calcium signals recorded simultaneously in M1 (medium), calcium signals in CA1, while the animal was freely behaving was also represented (lower). **b** Special standing posture occurred during CMS. **c** Mean signals (red and bold) reflecting on the kinetics of calcium flashing. **d** Spectrogram of calcium signals in CA1 recorded from normal freely moving mouse, extracted using STFT obtained via functions in MATLAB. **e** Spectrogram of CMS-related calcium signals in CA1 recorded after KA administration. Signals lower than red lines are main components of Ca^{2+} transients of CA1

was defined as slowly propagating calcium signals related to a near-complete depolarization of quite a number of brain neurons and astrocytes (Enger et al. 2015; Somjen 2001). The different calcium kinetic was shown by the faster rising slope ($P = 0.0324$) and shorter rise time ($P = 0.0393$) of CSD-like events in CA1 than those in M1 (Fig. 3b). Particularly, we observed that CSD-like events in CA1 often occurred following calcium build-up (Fig. 3a, lower) (Berdyyeva et al. 2016), but the duration of CSD-like events (106.2 ± 16.91 s) was significantly longer than calcium build-up (32.85 ± 8.71 s) before them ($P < 0.0001$; Fig. 3c). In contrast to the synchronism of calcium baseline-rising, the synchronism of CSD-like events was negated, because the number of CSD-like events detected in CA1 was much more than that in M1, and no synchronous relationship between CA1 and M1 existed. Notably, our results showed that those calcium build-up before CSD-like events usually lasted for a long time and was always accompanied with a rise of calcium signals in M1 (Fig. 3d, e). Our results suggested that the propagating features of KA-induced seizures varied in CA1 and M1.

Our results showed that 83% (15 out 18) of CSD-like events occurred with the specific head nod (Fig. 3d, e; upper; Online Resource 4), but the rise of calcium signals simultaneously recorded in M1 happened before the behavior of head nod, as the normalized ΔF/F of calcium signals in M1 showed (Fig. 3d, e; upper). Other seizure symptoms could also be observed, including motionless staring/slight trembling (33%, $n = 5$; stage 1), only clonus of forelimbs or tail rigidity (27%, $n = 4$; stage 3), as well as clonic or tonic–clonic seizures with loss of posture, falling or wild jumping (40%, $n = 6$; stage 4 or stage 5; Online Resource

5). Additionally, 16.7% (3 out 18) of CSD-like events were associated with clonic seizures independently but without head nod mentioned before (16%, $n=3$; stage 4).

The kinetic of calcium flashing and its relation to convulsive motor seizure

The third kind of typical calcium event that we observed was calcium flashing (Fig. 4a, upper), which was characterized by the epileptiform signature of steady and low event rate (Berdyyeva et al. 2016). Our results found that this kind of event often occurred ~ 30 min after KA injection, and often occurred after the CSD-like events. Convulsive motor seizures (CMS) were observed during this kind of calcium event (Fig. 4b; stage ≥ 3; Online Resource 6). The typical symptoms of CMS including long-lasting loss of posture, falling and/or wild jumping (Racine 1972). However, this kind of event was only detected in CA1 and was failure to be detected in M1 of all mice ($n=6$). Our results showed that flashing events during seizures often happened with a stable rate (0.37 ± 0.07 Hz), and the duration of flashing events in CA1 (0.12 ± 0.01 s) was significantly shorter than CSD-like events ($P < 0.001$; Fig. 4c).

In addition, based on the complex high frequency of CMS calculated by power spectral density during KA-induced status epileptics (SE) (Berdyyeva et al. 2016; Tse et al. 2014), we used short-time Fourier transform (STFT), an efficient method to study cellular activities (Samiee et al. 2015; Tse et al. 2014; Wang et al. 2018), to explore the relationship between calcium signals and CMS behaviors. Fortunately, our results of time–frequency analysis using STFT indicated that the frequency band of the main energy distribution of calcium flashing in CA1 during CMS was higher than that in the physiological state (< 5 Hz; Fig. 4d, e). Therefore, our result suggested that STFT was useful for the judgement of abnormal calcium excitation and seizure CMS-like behaviors.

Discussion

As a typical multi-factorial syndrome of brain dysfunction, epilepsy has caused serious social burden worldwide (Singh and Trevick 2016). The epileptogenesis is thought to be a consequence of functional imbalance between brain cellular excitation and inhibition for persistently increased neuronal excitability (Delorenzo et al. 2005). Specially, cellular mechanisms and calcium homeostasis play an important role in the induction of seizure behaviors (DeLorenzo et al. 2006; Ugawa 2013). The characteristics of epileptic seizures induced by KA-administrated animal model are very close to the seizures of human temporal lobe epilepsy (Jiruska et al. 2013). Hippocampus is considered to be an important agitation point for epileptic seizure in KA-induced epilepsy, because it is rich in KA receptors (Bayat et al. 2017; Connell et al. 2017). The alterations of cellular calcium in primary motor cortex are closely related to the changes of behavior activities (Khoshkhoo et al. 2017; Zhang et al. 2017a). In this study, we simultaneously recorded the alterations of calcium signals in both hippocampal CA1 and primary motor cortex M1 to observe the possible synchronization of calcium events between the two brain areas and their relations to seizure-like behaviors in a freely moving KA-induced epileptic animal model by using multi-channel fiber photometry.

In our previous research and other studies, three calcium signal events were observed as fast flashing, similar to baseline rising in this study, CSD and slow flashing in hippocampus in freely moving epileptic mice (Berdyyeva et al. 2016; Zhang et al. 2019). In our previous study, we found that there were some stereotypical events of epileptiform calcium signals recorded separately in different brain regions such as the hippocampus, dentate gyrus and entorhinal cortex in freely moving mice (Zhang et al. 2019). The results imply that some kinds of relations of calcium signal events among different brain regions may exist in epilepsy. Multiple-channel simultaneous calcium signal recording system could be used to study the relationship of epileptic calcium events between different brain regions. But unfortunately, there is no epileptic related research yet, although the multiple-channel simultaneous calcium signal recording system had been used for other researches, even used for simultaneous recording of different animals (Allen et al. 2017).

Epileptic activity is considered to be associated with excessive neuronal excitability and their synchronization (Delorenzo et al. 2005; Francis et al. 2003; Jiruska et al. 2013). Furthermore, excessive calcium influx is directly to the excessive neuronal excitability in epileptic seizure (Delorenzo et al. 2005). It has still been unclear if there is any synchronous relationship due to lack of research that involves the simultaneous recording of calcium alteration representing the excessive neuronal excitability between two or more different brain regions. In this study, we observed three calcium events, baseline-rising, CSD-like events and flashing in hippocampal CA1 in KA-induced epileptic freely moving animals using a multi-channel fiber photometry system, which were similar to the three events, fast flashing/build-up, CSD and slow flashing observed in hippocampal CA1 respectively by Berdyyeva et al. using both EEG and micro-endoscopic calcium imaging (Berdyyeva et al. 2016). Meanwhile, we only simultaneously observed two kinds of calcium events, baseline-rising and CSD-like events, in primary motor cortex M1 in the same experiment with the multi-channel fiber photometry. We did find that the calcium event-baseline rising was well synchronous between hippocampal CA1 and primary motor cortex M1, but we did

not find any synchronous relation of the calcium CSD-like event between the two brain regions. Also, we were unable to determine the synchronous relationship of the calcium flashing between CA1 and M1, because this kind of stereotypical event could not be clearly identified in M1.

Baseline-rising event, similar to the fast flashing recorded in the EEG (Berdyyeva et al. 2016) appeared early, usually 5 min and recurrence later after KA administration to the mice. The co-occurrence of this simultaneous calcium events recorded in both CA1 and M1 confirms that the synchronization of epileptiform calcium signals between two or more brain regions exists in KA-induced epileptic animal model. CSD is a self-propagating wave of brain cellular depolarization that has been implicated in migraine and other progressive neuronal injury, such as stroke and head trauma at the speed of 2–6 mm/min (Enger et al. 2015). CSD was also observed as a distinguishing calcium event in epileptic animal models (Khoshkhoo et al. 2017). In this study, we observed CSD-like events from both neurons and glia labeled (Li et al. 2017) by Cal-520AM in CA1 and M1 using the multi-channel fiber photometry, but there was no synchronous relation of those CSD-like events between the two brain regions in the epileptic model. Our result suggests that the induction and propagation of CSD may limit within special brain regions and CSD-like events cannot spread over from the hippocampus to other brain regions.

Since calcium events were recorded simultaneously in both CA1 and M1 with the multi-channel fiber photometry in epileptic freely moving mice, it was easy for us to observe seizure-like behaviors in freely moving mice and analyze the relationship between epileptiform calcium events and seizure-like behaviors. We found that clonus of forelimbs/trembling (stage 3) (Racine 1972) occurred with the synchronous calcium baseline-rising events recorded in both CA1 and M1, which started in the early period of KA administration and reoccurrence later. Meanwhile, drastic convulsive motor seizures (stage 4, 5 according to Racine Scale) were observed with flashing events only in CA1, which started usually ~ 30 min after KA injection and lasted for a long time. Interestingly, unlike other studies, we found that the CSD-like events observed in CA1 were highly related to the head movement (especially as head nod) in the freely moving epileptic mice. The head movement may be caused by epileptic seizures, or it may also possibly be caused by CSD-related migraine, since CSD is widely considered to correlate with the aura preceding migraine (Enger et al. 2015).

Conclusion

We confirmed in this study that the synchronization of calcium baseline-rising events existed between two brain regions, but CSD did not show the synchronization and seemed spreading only within its own region in KA induced freely moving epileptic animal model with multi-channel fiber photometry. The epileptiform calcium flashing only observed in CA1 and another two calcium events simultaneously recorded in both brain regions in this study were associated with different seizure-like behaviors. CSD-related head movement may be caused by epileptic seizures or migraine. Our results may provide clues for further researches associated with epilepsy pathogenesis and clinical prevention and treatment.

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Author contributions All authors contributed sufficiently for being credited as an author for this article. XD, XZ, NNL, FFW and HS: conceptualization, methodology, investigation. XD, XZ, FFW, NNL, YYL, LPW, SYY, FC, SWH, QYJ, QH, CLG, TRW and HS: formal analysis, Data Curation, Software. XD and XZ: writing—original draft preparation. HS: writing—reviewing and editing, funding acquisition and supervision.

Compliance with ethical standards

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

Authorship XD and XZ should be considered joint first author.

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