Different Effects of Monophasic Pulses and Biphasic Pulses Applied by a Bipolar Stimulation Electrode in the Rat Hippocampal CA1 Region

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Research

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Abstract

Background: Deep brain stimulation (DBS) has been successfully used for treating certain brain diseases such as movement disorders. High-frequency stimulations (HFS) of charge-balanced biphasic pulses have been used in clinic DBS to minimize the risk of tissue damages caused by the electrical stimulations, while HFS sequences of monophasic pulses have been used in animal experiments to investigate DBS therapy. However, it is not clear whether HFS sequences of monophasic pulses could induce abnormal neuronal responses different from biphasic pulses. Thus, the present study investigates the differences of neuronal responses to HFS of monophasic pulses and biphasic pulses.

Methods: Orthodromic-HFS (O-HFS) and antidromic-HFS (A-HFS) of the two types of pulses (with a 1-min duration) were delivered by bipolar electrodes to the Schaffer collaterals (i.e., afferent fibers) and the alveus fibers (i.e., efferent fibers) of the rat hippocampal CA1 region *in-vivo*, respectively. Responses of CA1 pyramidal neurons to the stimulations were recorded in the CA1 region. Single pulses of antidromic- and orthodromic-test stimuli were applied before and after HFS to evoke population spikes for evaluating the baseline and the recovery of neuronal activity.

Results: Spreading depression (SD) appeared during sequences of 200 Hz monophasic O-HFS with a high incidence (4/5), but did not appear during corresponding 200 Hz biphasic O-HFS (0/6). The potential waveform of SD was accompanied by a preceding burst of population spikes, propagated slowly, silenced neuronal firing temporarily and resulted in a non-recovery of orthodromically-evoked population spikes (OPS) after the O-HFS. No SD events appeared during the O-HFS with a lower frequency of 100 Hz of monophasic and biphasic pulses (0/5 and 0/6, respectively) nor during the A-HFS of 200 Hz pulses (0/9). However, the antidromically-evoked population spikes (APS) only recovered partially after the 200 Hz A-HFS of monophasic pulses.

Conclusions: The O-HFS with a high enough frequency of monophasic pulses may induce the abnormal neuron activity of SD instantaneously, which may be used as a biomarker to warn the damages caused by improper stimulations in brain tissues.

Background

Electrical pulses have been used in deep brain stimulation (DBS) for treating certain neurological disorders successfully (e.g., Parkinson's disease and epilepsy) and for treating psychiatric disorders promisingly (e.g., major depression and obsessive compulsive disorder) [1, 2]. The DBS commonly utilizes sequences of narrow pulses with a pulse width around 0.1 ms and a pulse frequency ~ 100–200 Hz (termed as high-frequency stimulation, HFS). Charge-balanced biphasic pulses (with a preceding negative pulse immediately followed by a positive one) are usually utilized in clinical DBS for safety [3, 4], while negative monophasic pulses are used in animal studies for investigating DBS therapy [5–7]. However, it is not clear whether HFS sequences of monophasic pulses could induce abnormal neuronal responses different from biphasic pulses.

In theory, a negative pulse is more efficient to activate neurons than a positive pulse or a biphasic pulse in the situation of extracellular stimulation as DBS [8, 9]. However, continuous stimulations of negative monophasic pulses have a risk of tissue damage caused by irreversible chemical reactions, whereas biphasic pulses do not [8, 10]. The reverse electric field generated by the subsequent positive phase of a biphasic pulse may prevent an accumulation of cation/anion ions thereby preventing irreversible chemical reactions in the brain tissue around the electrode contacts. On the contrary, sequences of monophasic negative pulses could result in irreversible chemical reactions with toxic products to damage the brain tissue [8].

Besides poisons of electrochemical reactions, stimulation-related tissue damages may be also caused by other factors, such as depletion of oxygen by hyperactivity of neuronal firing, substantial changes of ionic concentrations in both intracellular and extracellular environments, and excitotoxicity caused by excessive release of glutamates [8, 11]. Some of the changes may lead to abnormal neuronal activity, such as a large shift of depolarization potential in brain regions, termed as spreading depression (SD) [12]. Thus, to investigate the differences of neuronal responses to HFS of monophasic pulses and biphasic pulses, we recorded the electrical signals in the hippocampal CA1 region in rat brain *in-vivo* during HFS of axonal fibers and observed the neuronal responses including the incidence of SD events induced by HFS of the two types of pulses.

The hippocampal region of brain is prone to generate SD events under certain circumstances [13, 14]. And, axons are the most susceptible structure of neurons to the extracellular stimulation of electrical pulses [15, 16]. Therefore, the incidence of SD induced by axonal HFS in hippocampal CA1 region could be used as a sensitive index to distinguish the neuronal responses to different sequences of HFS. Results of the study may provide new information for appropriate usage of pulse stimulations in DBS investigations and in clinical applications.

Results

Spreading depression appeared during orthodromic-HFS with monophasic but not biphasic pulses and with a higher rather than a lower stimulation frequency

Electrical pulses of biphase and monophase were delivered through the concentric bipolar electrodes placed at the Schaffer collaterals for the orthodromic-HFS (O-HFS) and at the alveus fibers for the antidromic-HFS (A-HFS), respectively (Fig. 1a and 1b). For the stimulations of a single pulse with a same current intensity (Fig. 1c), the mean amplitude of orthodromically-evoked population spike (OPS) induced by a monophasic pulse (9.44 ± 1.25 mV, $n = 5$) was ~ 23% greater than that induced by a biphasic pulse (7.69 ± 1.74 mV, $n = 6$; t-test, $P < 0.05$). The result was consistent with the theory that a monophasic pulse is more efficient than a biphasic pulse in eliciting neuronal firing, because the second positive phase of a biphasic pulse may have a reverse effect to the first negative phase thereby reducing the ability of the negative pulse to activate neurons in a certain extent [8, 9].

During the O-HFS of 1-min 200 Hz biphasic pulses, OPS events only appeared in the initial several seconds of O-HFS (Fig. 2a). After the disappearance of OPS, multiple unit activity (MUA) continued to the end of the O-HFS with a firing rate of unit spikes higher than baseline. A silent period (10–30 s) without MUA appeared immediately following the end of O-HFS, indicating that the unit spikes during the O-HFS period were induced by the stimulation. During the period of O-HFS, an antidromic-test (A-test) pulse was applied every 5 seconds (i.e., 0.2 Hz) at the alveus fibers to evaluate the excitability of the CA1 neurons. The single A-test pulses and orthodromic-test (O-test) pulses were also applied before and after O-HFS to evaluate the baseline and the recovery of neuronal activity. Large antidromically-evoked population spike (APS) evoked by A-test pulses persisted throughout the 1-min O-HFS, and the mean amplitude of these APS (7.26 ± 5.59 mV, $n = 6$) was ~ 17% greater than the corresponding baseline level (6.19 ± 3.19 mV, $n = 6$; paired t-test, $P < 0.05$). These results indicated that the sustained O-HFS increased the excitability of the CA1 neurons. About 4 min after the end of O-HFS, both test APS and test OPS evoked by single pulses recovered to baseline level. In addition, no SD event appeared in all of the 6 rats with the 200 Hz biphasic O-HFS.

However, SD events appeared in 4 of the 5 rats with the 200 Hz monophasic O-HFS (Fig. 2b). The initial neuronal responses induced by the monophasic O-HFS was similar to that induced by the biphasic O-HFS: large OPS appeared at first, then OPS disappeared and dense MUA appeared. However, an SD appeared later with a slow waveform lasting 4.31 ± 3.11 s ($n = 4$). At the same time, the MUA disappeared completely and the A-test pulses were no longer able to induce APS, indicating a silence of neuronal activity. The MUA did not appear until 3.51 ± 2.47 min ($n = 4$) after the end of O-HFS (Fig. 2b, *bottom*). By this time, the test APS recovered to ~ 80% of baseline level. The test OPS did not recover even ~ 25 min after the end of O-HFS, while the test APS had almost recovered to baseline level (89.5 ± 9.7%, $n = 4$. Figure 2b, *middle*).

The 16 channels arranged in the four shanks of recording electrode (RE) showed the spread of SD waveforms (Fig. 3a). The waveforms of baseline OPS and APS along the shanks indicated the locations of each recording channel in the different stratums of CA1 region [17]. Because the signal recording in this study was AC-coupled (0.3–5000 Hz), the SD waveform appeared as a trough similar to previous reports [18]. The SD trough appeared first in the stratum radiatum (S. rad.) of hippocampal CA1 region (Fig. 3b), accompanied by a burst of population spikes (60–80 spikes/s) in the stratum pyramidale (S. pyr.) that was prominent in the filtered signals greater than 10 Hz (Fig. 3b *right*). Then the SD trough propagated slowly to the CA1 layers of S. pyr. and stratum oriens (S. ori.) at a speed of 89.9 ± 50.9 μm/s ($n = 4$) in the perpendicular direction, characterized by the movement of the negative peak of SD trough along the recording shanks (Fig. 3b, hollow triangles). Also, the SD trough moved at a speed of 826 ± 627 μm/s ($n = 4$) in the S. pyr. layer transversely among the recording shanks (Fig. 3a *right*, blue dotted line). The characteristics of the SD events, including the waveform, the accompanied burst of population spikes, the slow travelling speed and the silence of neuronal electrical activity, were consistent with previous reports [18, 19].

The result of statistical test showed that the SD incidence during 200 Hz monophasic O-HFS (4/5) was significantly greater than the incidence during 200 Hz biphasic O-HFS (0/6; Fisher's exact test, $P < 0.05$). In addition, with a decrease of the O-HFS frequency from 200 to 100 Hz, no SD events were observed with monophasic O-HFS (five rats) and with biphasic O-HFS (six rats). Therefore, given the data of monophasic O-HFS only, the SD incidence during 100 Hz O-HFS (0/5) was significantly lower than that during 200 Hz O-HFS (4/5; Fisher's exact test, $P < 0.05$).

These results indicated that O-HFS of monophasic pulses with a higher stimulation frequency may generate SD events in the hippocampal CA1 region and affect the orthodromic pathway persistently. The generation of OPS by the stimulation at afferent fibers involves both the axonal conductions and the synaptic transmissions. Therefore, the non-recovery of OPS after the monophasic O-HFS could have been caused by potential damages in the axons and/or synapses. To confirm whether the monophasic HFS could cause damages in axons, we next inspected the responses of CA1 neurons to the A-HFS at their own axons (i.e., the alveus) without involving synaptic transmissions.

More attenuation of population spike amplitudes during A-HFS of monophasic pulses

During the 1-min 200 Hz A-HFS of biphasic pulses, APS was able to follow each stimulation pulse with a large amplitude only at the initial period and then the APS amplitude decreased rapidly (Fig. 4a). Single test pulses showed that ~ 1 min after the end of A-HFS, the test APS recovered to ~ 70% of baseline level. And, ~ 2 min after the end of A-HFS, the test APS recovered to baseline level (92.2 ± 21.0%, $n = 4$).

When the A-HFS was applied with monophasic pulses, the neuronal responses at the initial period were similar to that of biphasic pulses – each pulse induced a large APS. However, at the late period of A-HFS, the monophasic pulses hardly induced APS (Fig. 4b). In addition, the test APS only recover to 34.5 ± 12.1% ($n = 5$) of baseline level even ~ 20 min after the end of A-HFS.

The APS amplitude decreased rapidly at the onset of A-HFS and became steady within several seconds and persisted until the end of A-HFS, which may be caused at least partially by the depolarization block of axons [5, 20]. We calculated the APS amplitudes normalized to baseline value during the A-HFS period to evaluate the changes of APS (Fig. 4c). The decrease of APS amplitudes at the initial 1 s (ΔA_{1s}) of A-HFS was significantly greater with monophasic A-HFS (86.9 ± 8.7%, $n = 5$) than with biphasic A-HFS (73.5 ± 8.0%, $n = 4$; t–test, $P < 0.05$. Figure 4d). Also, the decrease of APS amplitudes at the end of A-HFS (ΔA_{60s}) was significantly greater with monophasic A-HFS (98.6 ± 1.2%, $n = 5$) than with biphasic A-HFS (94.1 ± 3.4%, $n = 4$; t–test, $P < 0.05$. Figure 4e).

The partial recovery of test APS after monophasic A-HFS together with the faster and larger degree of APS attenuation during A-HFS indicated that the monophasic A-HFS may cause conduction failures in a portion of axons even after the A-HFS.

In addition, no SD event was observed in all the 9 rats applied 1-min 200 Hz A-HFS (4 with biphasic pulses and 5 with monophasic pulses). That is, the SD incidence during 200 Hz monophasic A-HFS (0/5)

was significantly smaller than that during 200 Hz monophasic O-HFS (4/5; Fisher's exact test, $P < 0.05$).

Discussion

The major findings of the present study in the rat hippocampal CA1 region include: O-HFS of monophasic pulses at the afferent fibers with an enough high frequency may induce spreading depression (SD) events and affect the pathway of orthodromic activation; and A-HFS of monophasic pulses at the efferent fibers may cause conduction failures in a portion of axons even after the A-HFS but not induce SD events. Possible mechanisms underlying the findings are discussed below.

SD is a type of large shift of depolarization potential in brain regions. The earliest discovery of SD was associated with epileptic bursts and was described as the suppression of electroencephalographic signals following bursts of seizure discharges in the cortex regions [21]. Studies have also shown that SD events may follow neuronal discharges in other brain regions, such as the hippocampus [18]. One of the major associated characteristics of SD is the great concentration changes of certain ions in the extracellular space, especially a substantial elevation of extracellular potassium concentration ($[K^+]_o$), indicating redistributions of the ions between extra- and intra-cellular spaces [22].

In the present study, SD events only appeared during monophasic O-HFS but not during biphasic O-HFS. Thus, certain effects associated with the HFS of monophasic pulses may be responsible for the appearance of SD. In contrary to the biphasic pulse, the monophasic pulse drives the ions in fixed directions in the brain tissue by the force of the unidirectional electric field. The cations migrate to the cathodic pole of the electrode and the anions to the anodic one. Under the action of repetitive monophasic pulses with a high frequency, the ions may accumulate around the two poles of the electrode and destroy the ionic equilibriums between the intracellular and extracellular environments. Previous studies have shown that repetitive stimulations can enhance $[K^+]_o$ in the CA1 region [23]. Presumably, the increase of $[K^+]_o$ may be exacerbated by the HFS of monophasic pulses because of the cationic accumulation by the unidirectional driving force around the small space of electrode poles (Fig. 1b). The high $[K^+]_o$ may result in a depolarization in neuronal membranes and generate epileptiform activity [24, 25], just as the burst of population spikes preceding SD trough in the present study (Fig. 3b). The burst activity may further elevate $[K^+]_o$ thereby resulting in the large shift of depolarization potential of SD. Given the fact that a potassium injection with a molar level of concentration can induce SD events in animal preparations [14, 26, 27], the accumulation of K^+ by monophasic pulses may be one of the mechanisms to generate SD.

In addition, synapse-related effects may also contribute to the generation of SD, because SD events didn't appear during A-HFS but appear during O-HFS involving synaptic transmissions. Previous studies have shown that an excessive concentration of glutamates released from excitatory synapses to the extracellular space can induce SD [27–29]. In the present study, the O-HFS in the afferent fibers can activate the excitatory synapses and cause glutamates releases at the S. rad., since the layer of apical

dendrites of pyramidal neurons (i.e., S. rad.) is full of glutamatergic synapses. In addition, the elevation of $[K^+]_o$ by monophasic O-HFS may reduce the uptake of glutamates, and reduce extracellular space by astrocytes swelling, thereby further increasing the concentration of extracellular glutamates [30, 31]. Thus, an elevation of glutamates caused by monophasic O-HFS may be another factor to generate SD.

Furthermore, the non-recovery of the test OPS after monophasic O-HFS and the partial recovery of APS after monophasic A-HFS suggested that the application of intensive monophasic pulses may damage the axons under the stimulations. As previous reports, the toxic chemical products of irreversible chemical reactions at the electrode-tissue interface by monophasic stimulations can damage the neuronal tissues. The reactions include electrolysis of water, oxidation of saline, metal, and organic materials, as well as reduction of oxygen [32]. In addition, according to mass action theory, an extreme excitation by over-stimulating the excitable tissue may result in the depletion of oxygen or/and glucose and substantially change both the intracellular and extracellular concentrations of ions [8]. These factors may result in neuronal dysfunction and tissue damages. Further histological and morphological analysis of stimulated tissues may confirm the tissue damages. Nevertheless, the appearance of SD may be a sensitive index to warn the tissue damages.

Taken together, an extreme elevation of $[K^+]_o$ and an increase of glutamate concentrations may result in the generation of SD by the monophasic O-HFS. In addition, the irreversible chemical reactions induced by the monophasic HFS with an enough high frequency may damage a portion of the axons in the stimulation site.

Conclusions

The comparisons of neuronal responses to the HFS of monophasic- and biphasic-pulses show that O-HFS with an enough high frequency of monophasic pulses may induce the abnormal neuron activity of spreading depression. The SD event may be used as a biomarker to warn the damages caused by improper stimulations in brain tissues instantaneously.

Methods

Surgical procedures

The animal experiment was approved by the Institutional Animal Care and Use Committee, Zhejiang University. Twenty adult male Sprague-Dawley rats were used: 11 for O-HFS (6 for biphasic and 5 for monophasic) and 9 for A-HFS (4 for biphasic and 5 for monophasic). Each rat was anesthetized with urethane (1.25 g/kg, i.p.) and was placed in a stereotaxic apparatus (Stoelting Co.). Details of the surgery and the electrode placements were similar to the previous report [33]. Briefly, a 16-channel recording electrode array (#A4 × 4-3mm50-125-177, NeuroNexus Technologies, USA) was inserted into the hippocampal CA1 region to record electrical potentials. Two concentric bipolar stimulation electrodes (#CBBRC75, FHC Inc., USA) were positioned in the Schaffer collaterals and in the alveus fibers of CA1

region for orthodromic- and antidromic-HFS, respectively (Fig. 1a). The distance between the inner and outer poles of the stimulation electrodes was short (< 100 μm) (Fig. 1b), indicating a small environment around the two poles. The signals of multiple unit activity (MUA) and the waveforms of both orthodromically-evoked population spike (OPS) and antidromically-evoked population spike (APS) along the recording channels were used to guide the final positions of the three electrodes.

Stimulation And Recording

Sequences of current pulses of monophase (negative phase) and biphase (a preceding negative phase followed by a positive phase) with a phase width of 0.1 ms (Fig. 1c) were generated by a stimulator (Model 3800, A-M Systems, Inc. USA). The current intensity was 0.3–0.5 mA that was able to induce a population spike (PS) with an amplitude approximately 75% of the maximal PS amplitude. The pulse frequency was 100 or 200 Hz for O-HFS at the Schaffer collaterals, and 200 Hz for A-HFS at the alveus fibers of the hippocampal CA1 region. The duration was 1 min for both O- and A-HFS.

Recording signals were amplified 100 times by an AC-coupled (0.3–5000 Hz) amplifier (Model 3600, A-M Systems, Inc. USA). The amplified signals were then sampled at a rate of 20 kHz by a Powerlab data acquisition system (ADInstruments, Inc. Australia).

Data analysis

The artifacts of stimulation pulses in the raw recording signals were removed by an algorithm to detect each artifact segment (~ 1.0 ms) which was then replaced by an interpolation line [34]. The artifact-free signals were then filtered by digital high-pass filters with a cut-off frequency of 500 Hz to obtain the MUA signals, or with a cut-off frequency of 10 Hz to remove the slow waveform of SD to illustrate the burst population spikes accompanying SD. The recording signals from the channel closest to the cell body layer (i.e., S. pyr.) were used to calculate the amplitudes of evoked-PS.

Data were expressed as mean ± standard deviation and *n* represented the number of rat experiments. Statistical Fisher's exact test and *t*-test were used to judge the significance of differences among data groups.

Abbreviations

DBS
deep brain stimulation; HFS:high-frequency stimulation; SD:spreading depression; OPS:orthodromically-evoked population spikes; APS:antidromically-evoked population spikes; MUA:multiple unit activity; S. rad.:stratum radiatum; S. pyr.:stratum pyramidale; S. ori.:stratum oriens.

Declarations

Ethics approval and consent to participate:

This study was carried out in accordance with the recommendations of Guide for the Care and Use of Laboratory Animals by China Ministry of Health. The protocol was approved by the Institutional Animal Care and Use Committee, Zhejiang University, Hangzhou.

Consent for publication:

Not applicable.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

ZF, YY and LZ conceived and designed the experiments. YY, LZ and GY performed the experiments. ZF, YY and LZ analyzed and interpreted the data, and wrote the paper. All authors read and approved the final manuscript.

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Figures

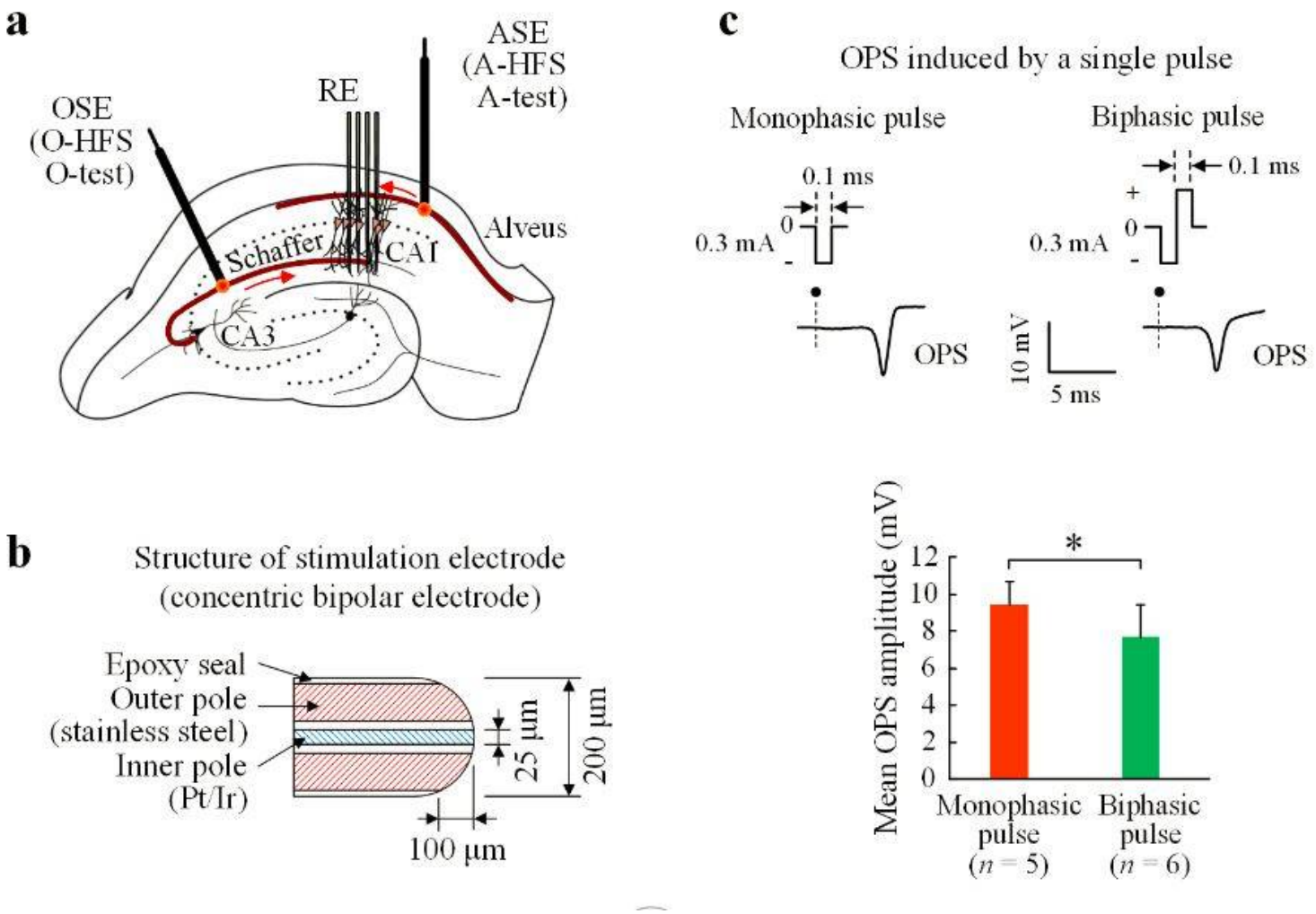

Figure 1

Schematic diagram of electrode locations, electrode geometry and waveforms of evoked potentials. a Recording electrode (RE) in the hippocampal CA1 region, and stimulation electrodes for orthodromic activation (OSE) and antidromic activation (ASE) in the Schaffer collaterals and alveus fibers, respectively. b The tip of the concentric bipolar stimulation electrode. c Top: Examples of orthodromical population spike (OPS) evoked by monophasic and biphasic pulses. Bottom: Comparison of mean amplitudes of baseline OPS induced by single pulses of monophase (n = 5) and biphase (n = 6) with a same current intensity. *P < 0.05, t-test.

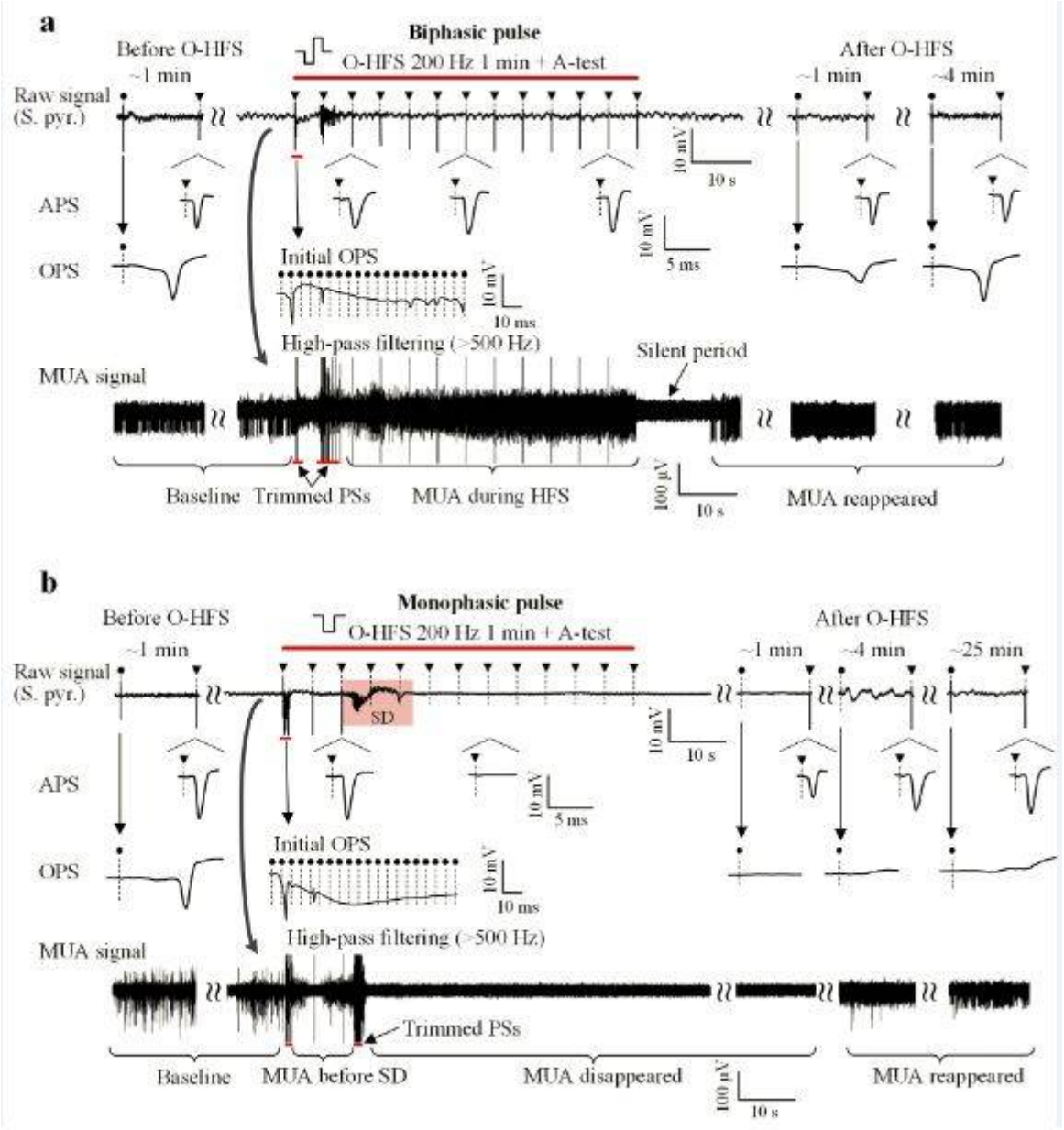

Figure 2

Spreading depression induced by 200 Hz O-HFS of monophasic pulses in the hippocampal CA1 region. Typical examples of recording signals in the CA1 pyramidal layer with 1-min 200 Hz O-HFS (denoted by the red bar) of biphasic pulses (a) or monophasic pulses (b). The pink shade in (b) denotes an SD event. The APS and OPS waveforms induced by A-test pulses at the alveus and by O-test pulses at the Schaffer collaterals in the periods before, during and after O-HFS are expanded. The small triangle with a dotted line denotes an antidromic pulse and the dot with a dotted line denotes an antidromic pulse. The MUA signals in the periods before, during and after O-HFS were obtained by filtering the raw signals.

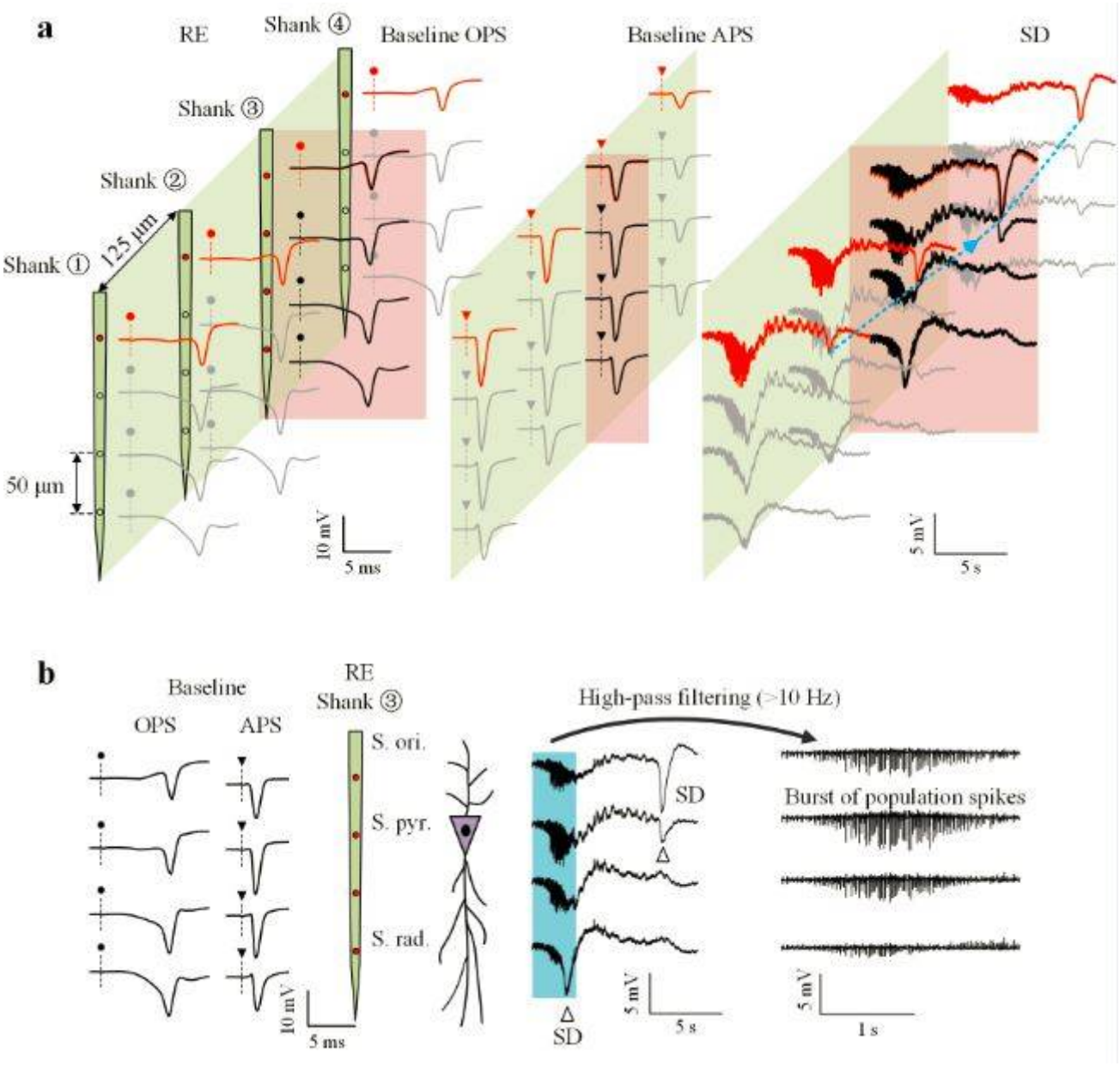

Figure 3

Propagation of an SD waveform. a Signals recorded by the four parallel shanks of the 16-channel RE array. Left and middle: baseline 16-channel OPS and APS waveforms evoked by a single pulse. Right: an SD event in the 16-channel recordings. The blue dotted line denotes the movement of the SD trough in the horizontal direction. b The four channels of recording signals along the third shank of RE denoted the pink shade in (a). The waveforms of baseline OPS and APS indicate the locations of each recording channel in the different stratums of CA1 region. The hollow triangles denote the movement of the SD trough in the perpendicular direction. The blue shade denotes the burst of population spikes at the onset of SD that are filtered and expanded on the right.

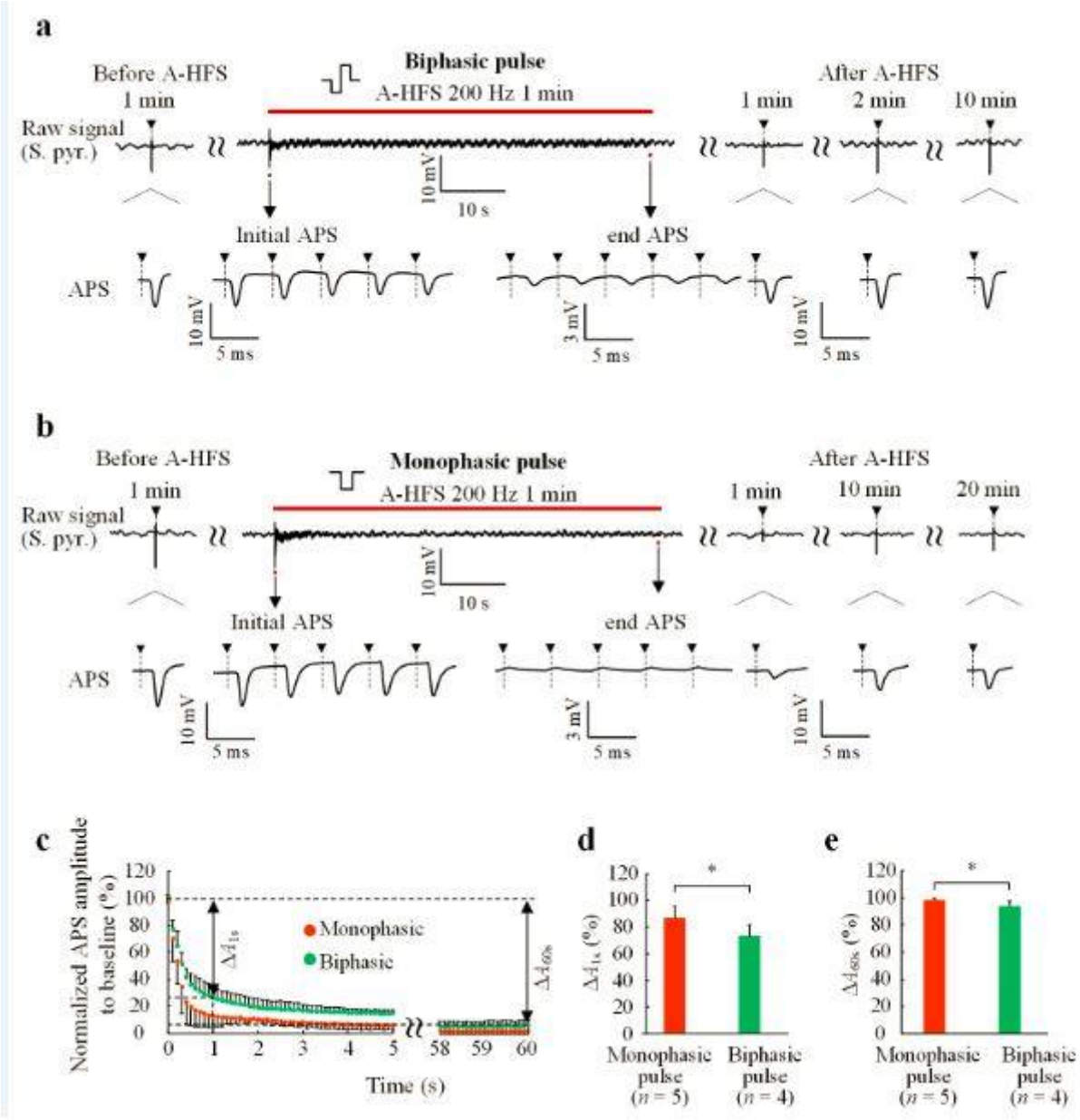

Figure 4

Neuronal responses induced by 200 Hz A-HFS in the hippocampal CA1 region. a and b Top: Typical examples of recording signals in the S. pyr. with 1-min 200 Hz A-HFS (denoted by the red bar) of biphasic pulses (a) or monophasic pulses (b). Bottom: Typical waveforms of APS induced by A-test pulses or A-HFS pulses in the periods before, during and after A-HFS. c Normalized APS amplitude in the first 5 s and last 2 s of A-HFS. d and e: Comparisons of the decreases of APS amplitudes at 1 s (ΔA1s) and at 60 s (ΔA60s) by monophasic A-HFS (n = 5) and biphasic A-HFS (n = 4). *P < 0.05, t-test.