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Functional Disconnection of Axonal Fibers Generated by High Frequency Stimulation in the Hippocampal CA1 Region *in-vivo*

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Abstract

High frequency stimulation (HFS) applied in the brain has been shown to be efficient for treating several brain disorders, such as Parkinson's disease and epilepsy. But the mechanisms underlying HFS are not clear. In particular, the effect of HFS on axons has been intriguing since axonal propagation plays an important role in the functional outcome of HFS. In order to study the potential effect of axonal block by HFS *in-vivo*, both orthodromic- and antidromic-HFS (O-HFS, A-HFS) with biphasic pulses were applied to the hippocampal CA1 region in anaesthetized rats. Changes in the amplitude and latency of orthodromic- and antidromic-population spikes within 1-min long period of stimulation at 50, 100 and 200 Hz HFS were analyzed. The results show that except for a short onset period, activity evoked by O-HFS with a frequency over 100 Hz could not be sustained. For A-HFS at frequencies over 100 Hz, the amplitudes and the latencies of antidromic population spike (APS) greatly decreased and increased respectively. Significantly larger changes in APS latency were observed in HFS than those generated at low frequency, suggesting a suppression of axon conduction by HFS. Furthermore, the CA1 somata remained excitable while failing to respond to excitation from orthodromic or antidromic HFS. Taken together, these results show that HFS produces an axonal block of both afferent and efferent fibres localized to the area of stimulation since it does not affect the excitability of CA1 somata. This effect of HFS on axons causes a functional disconnection of axonal pathways that is reversible and temporary. The reversible disconnection or temporary deafferentation between putative therapeutic targets could have extensive implication for various clinical applications of HFS to treat brain diseases.

Keywords

High frequency stimulation; Hippocampus; Axonal block; Evoked potential; Biphasic pulses

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1. Introduction

Deep brain stimulations (DBS) have been extensively investigated for treating various brain disorders in clinic, such as Parkinson's disease, epilepsy, pain, addiction, stroke, obsessive-compulsive disorder and depression (Awan et al., 2009; Hamani et al., 2009; Kuhn 2011; Datta et al., 2011; Mayberg et al., 2005). One possible mechanism of DBS is that it generates some depressive effects to prevent neurons from over-firings, at least in some applications. The major suppression mechanisms of DBS that have been proposed include: enhancing the actions of pre-synaptic inhibition by stimulation, hyperpolarizing local neuronal somas and dendrites; as well as depolarization blockage and neurotransmitter depletion (Mayberg et al., 2005; Deniau et al., 2010; Montgomery and Gale, 2008). However, a possible suppression effect of DBS on axons has generally been ignored.

Studies of the efficacy of clinical DBS treatment have shown that persistent high-frequency stimulation (HFS) over 100 Hz has been effective for controlling hippocampal epilepsy as well as Parkinsonian tremor (Velasco et al., 2007; Jobst 2010; Boon et al., 2007; Montgomery and Gale 2008). Axons have a low-pass filtering feature and can only conduct signals faithfully with a large inter-spike interval of ~90 ms (Chomiak and Hu, 2007; Bucher and Goaillard, 2011). It is unlikely that central axons can follow high frequencies faithfully. They will therefore present failures within long episodes of HFS.

However, there are contradictory views on the axonal block caused by HFS. Many investigators have considered that axonal suppression induced by DBS to be unlikely (Montgomery and Gale, 2008). Previous in-vitro recordings of ventral thalamic neurons did show that axonal HFS could cause a so called "functional deafferentation" of postsynaptic targets. But the mechanism was presumed to be resulting from transmitter depletion of synapses, not axonal block (Anderson et al. 2006; Iremonger et al. 2006). Other investigators have shown some evidence of block of axonal conduction by HFS in the hippocampus and subthalamus (Jensen and Durand, 2007, 2009; Zheng et al., 2011). However, most experiments were carried out in-vitro brain slices. Evidence from in-vivo preparations is essential to test the hypothesis that HFS can generate axonal block.

In order to test this hypothesis, we applied both orthodromic- and antidromic-HFS with biphasic pulses in the hippocampal CA1 region in anaesthetized rats. During 1-min long trains of HFS with frequency over 100 Hz, substantial neuronal firing activity was present only for a transient-period at the beginning of HFS, and followed by a period with little or no population spike activity. The changes in axon conduction and in soma excitability during the HFS were investigated by combining orthodromic- with antidromic-stimulation. The results support the hypothesis that axons can be blocked during HFS.

2 Results

2.1 Neuronal activity during HFS trains

In order to determine the effect of HFS applied to either afferent fibers or efferent fibers on evoked neural activity, we placed orthodromic- and antidromic-stimulation electrodes (OSE and ASE) in the Schaffer collateral and alveus of CA1 region, respectively (Fig.1A). Baseline recordings of evoked potentials were used to confirm the correct placements of the electrodes (Kloosterman et al., 2001). The first stimulus of a paired-pulse in the afferent Schaffer collaterals could induce an orthodromic population spike (OPS) with large amplitude. But, after a short inter-pulse interval of 50 ms, the second stimulus did not induce any noticeable OPS as expected from the inhibitory activity of GABAergic interneuron networks (Alger and Nicoll, 1982; Leung et al., 2008). However, the identical paired-pulse stimulus applied to the alveus could induce two antidromical population spikes (APS) with

similar large amplitudes. Although antidromic stimulation in the alveus could simultaneously activate the inter-neurons in the feed-back inhibition network, activating neurons by antidromic pathway does not involve the synaptic potentials. Antidromic stimulation initiates action potential in the axon initial segment directly, rather than in the cell body or dendrite as orthodromic stimulation (Kloosterman et al., 2001). Therefore, there was no paired-pulse depression in the APS.

During 1-min long 100 Hz HFS trains, CA1 neurons also responded differently to orthodromic-HFS (O-HFS) and antidromic-HFS (A-HFS). In a typical response to O-HFS (Fig 1B), the first stimulation pulse produced an initial large OPS, but the following several pulses failed to evoke large responses due to the GABAergic inhibition (Leung et al., 2008). After ~70 ms, the PSs reappeared but did not pace with every stimulation pulse (see the first expanded inset in Fig 1B). The PSs then disappeared again and did not reappear for the remainder of the stimulation period.

A typical response to A-HFS is shown in Fig 1C. APSs remained synchronized with the stimulation pulses but their amplitudes declined rapidly within the first seconds of HFS. Small APSs remained until the end of stimulation period (Fig 1C, time expanded insets).

HFS applied with different stimulation frequencies induced variable responses with higher frequencies generating much fewer PSs (in O-HFS) or much smaller PSs (in A-HFS) compared to lower frequencies (See 200Hz HFS compared to 50Hz stimulation in Fig 2). For higher frequency O-HFS, PSs appeared only at the initial period of HFS and their amplitude changed irregularly (see Fig 1B). Therefore, we defined an index, T_{PS} , to quantify the time extent with PS activity during O-HFS (see definition in Fig 2A). However, during A-HFS, PSs followed every pulse and their amplitudes declined regularly. Therefore, we used a different index, R_{PS} , defined as the amplitude ratio of the last APS to the first APS to describe the change of PS activity (see Fig 2B). Table 1 shows the statistical results of the experiments.

With similar stimulation intensity and similar initially evoked-PSs, T_{PS} values of 100 and 200 Hz O-HFS were significantly shorter than that obtained from 50 Hz O-HFS (Post hoc Bonferroni test $P < 0.001$). Except for the PS activity at the onset of O-HFS, neurons failed to follow most stimulation pulses with frequency over 100 Hz. For the A-HFS, at the end of 1-min long period of stimulation, the average amplitudes of PS were suppressed by more than 80% and 90% (i.e., 100%) - R_{PS} in Table 1) for 100 and 200 Hz A-HFS, respectively. They were both significantly smaller than the PS amplitude of 50 Hz A-HFS (Post hoc Bonferroni test $P < 0.001$). Although the values of T_{PS} and R_{PS} of 200 Hz were both smaller than those of 100 Hz, the differences were not statistically significant (Post hoc Bonferroni test $P > 0.06$, Table 1).

These results indicate that HFS at frequencies greater than 100Hz cannot produce sustained evoked potentials. This effect could be caused by failures in neuronal axons, synapses or somata. In order to study the mechanisms, we firstly investigated the neuronal response to the A-HFS.

2.2 Axonal suppression induced by antidromic HFS

The effect of synaptic transmission on the response to HFS could be eliminated by investigating the evoked-potential induced by A-HFS. Within a 200 Hz A-HFS train, almost every pulse could induce an APS response; however, at the onset of the train, the amplitudes of APSs decreased while their latencies increased, rapidly. Then, the two measurements waggled within small ranges of ~1 mV and ~1.2 ms, respectively (Fig 3A). The time expanded data of the first 1 s period of HFS show that the PS amplitudes decreased from 9.8

to 2.1 mV (“ Δ Amplitude” = 7.7 mV) while the APS latencies increased from 1.5 to 2.6 ms (“ Δ Latency” = 1.1 ms, Fig 3B).

Given the constant stimulation current, both the amplitude decrease and the latency increase in APSs could be caused by either a decreased excitability of pyramidal cell bodies, or a conduction failure of alvear axons in the CA1 region. In order to determine which of these two mechanisms could explain the effect of HFS, the changes of APS latencies in the I/O curves generated at very low frequency (< 0.05 Hz) were used as a reference and compared to the changes of APS latencies at the onset of HFS. In spite of large changes in the APS amplitudes (“ Δ Amplitude” = 6.3 ± 1.6 mV, $n = 16$), the changes in APS latencies were very small in the I/O curves (“ Δ Latency” = 0.08 ± 0.07 ms, $n = 16$, Fig 4). These results indicate that at low stimulation frequency, the conduction speed of the axons remains constant.

In contrast to the changes generated at low frequency, high frequency pulses (50, 100 and 200 Hz) at the onset of A-HFS generated much smaller changes of APS amplitude and were accompanied by much larger changes of latencies (Fig 4B). For example, “ Δ Amplitude” was 1.8 ± 1.4 mV and “ Δ Latency” was 0.36 ± 0.11 ms ($n = 10$) at 51th stimulus (i.e. 0.5 s) of 100 Hz HFS. Because neuronal cells can normally fire at high frequency for longer period of time as observed during after-discharges (Bragin, 1997; Lesser, 1999), the prolonged latencies of APS in such short time duration at the onset of A-HFS could not primarily result from the excitability decline of neuronal body, but could be caused by a decrease of conduction velocity in alvear axons.

Presumably, O-HFS in the Schaffer collaterals of CA1 afferent fibers could also induce axonal conduction failures similar to A-HFS of the alveus, thereby inducing long “silent period” without PS activity at higher frequencies of 100 and 200 Hz HFS as shown in Fig 1B, Fig 2A and Table 1. If the hypothesis of axonal block was correct, then the somata of downstream cells should have retained their capability to fire even during the “silent period” of O-HFS. Therefore, an antidromic test stimulus should be able to evoke a large APS in the period. The hypothesis was tested next.

2.3 Excitability of pyramidal cells during HFS of afferent and efferent axons

To test the hypothesis that the excitability of the CA1 pyramidal cells is not eliminated by O-HFS of the afferent axons, antidromic test stimuli (ATS) were applied every 5 s during O-HFS with the first ATS set at a delay of 20 ms after the first pulse of the O-HFS. In a total of four 100 Hz and six 200 Hz O-HFS experiments, the first test APS was suppressed by the large first OPS. However, the amplitude of other subsequent test APSs were even larger than baseline APSs, while the HFS-evoked OPSs were totally suppressed (see the sample recording in Fig 5A and the statistical results in Fig 5B). Following the O-HFS, antidromic and orthodromic test stimuli were applied every 1 min. The OPSs gradually recovered in ~7 min while APSs returned to the baseline level. (For comparison, during the HFS, only samples of OPS taken every 5 s were shown in the Figure 5B.) This result indicates that the suppression of OPS during O-HFS could not be attributed to an excitability decrease of the post-synaptic cells. Actually, the excitability of post-synaptic neurons could be even higher than baseline level since the amplitude of test APS increased. Presumably, during O-HFS period, continuous sub-threshold inputs in the afferent axons could still remain since the axon was not totally blocked (See Fig 1C and Fig 3). They might increase the excitability of post-synaptic neurons although they failed to induce OPS with obvious amplitude. However, the experiments of O-HFS can only suggest a pre-synaptic mechanism, but can not distinguish axonal failure from synaptic failure.

Next, experiments of A-HFS were carried out to eliminate the possibility that synaptic failure was a major cause of the suppression. In a reverse method to the above O-HFS

design in Fig 5, during A-HFS of efferent axons (alveus), orthodromic test stimuli (OTS) were applied every 5 s with the first OTS set at a delay of 20 ms after the first pulse of the A-HFS (Fig. 6A). In a total of four 100 Hz and five 200 Hz A-HFS experiments, the HFS-evoked APSs were obviously suppressed to amplitudes lower than 20% of baseline values, as expected. However, neither the amplitude of the OTS-evoked PS potentials nor the slope of the dentritic population excitatory post-synaptic potentials (fEPSP) changed markedly during the A-HFS, except that the first test OPS was suppressed by the large APSs at the onset of A-HFS (see the sample recording in Fig 6A and the statistical results in Fig 6B). Following A-HFS, the APSs recovered within ~4 min while OPSs gradually returned to the baseline level.

These results show that the CA1 pyramidal cells could still fire synchronously in response to low frequency orthodromic stimulation while they failed to respond to high frequency antidromic stimulation. This indicates that the cell bodies did not lose excitability. Since the antidromic pathway involves only axons and cell bodies (no synapse), axonal block is necessary and sufficient to explain the failures of antidromic-HFS (Fig. 6). Because of the bi-directional conduction feature of axons, these data strongly suggest that the orthodromic response suppression by O-HFS (Fig 5) is also generated by an axonal block. Therefore, axonal block could be a major mechanism of the suppression event in both antidromic and orthodromic-HFS.

3 Discussion

The major findings in this study include: 1) High frequency stimulation of either antidromic or orthodromic axonal pathways could not induce persistent population spikes with a frequency over 100 Hz in the hippocampal CA1 region. 2) HFS induced a marked decrease in conduction velocity of the axons. 3) The CA1 somata remained excitable while they failed to respond to excitation from orthodromic or antidromic HFS. The axonal block mechanism underlying these observations is analyzed below.

Axonal block during HFS

There is little information available in the literature on the effect of stimulation on neural activity within long periods of HFS since the high amplitude artifacts can mask the neural activity (Lee et al., 2010). In the above study we used a custom-made MATLAB program to remove the stimulation artifacts in the recording signals. As a result, it was observed during in-vivo experiments that the activity evoked by HFS of the CA1 afferent axons was suppressed during long “silent periods”. Three pieces of evidence support an axonal block mechanism to explain this suppression: 1) the speed of axonal conduction decreased markedly, 2) the firing ability of post-synaptic cells remained intact while they were “silent” to the orthodromic HFS, and 3) neurons were still able to respond to orthodromic stimulation while ignoring the excitation from continuous antidromic HFS.

First, it has been shown that neurons can normally fire for long period of time lasting several minutes during epileptiform activity (Bragin, 1997; Lesser, 1999). Also, population spikes could persist during 1-min long 50 Hz HFS in the present data (see Fig 2A and Table 1). Therefore, the marked decrease of APS amplitudes and increase of APS latencies that appeared within a short time window ~ 0.5 s at the onset of A-HFS over 100 Hz (see Fig 3 and Fig 4) could not be explained by an inability of the cells to fire at high frequencies.

Second, previous in-vitro recordings of ventral thalamic neurons actually have shown that axonal HFS could produce a functional deafferentation of postsynaptic targets, and the mechanism was presumed to be the result of transmitter depletion of synapses (Anderson et al. 2006; Iremonger et al. 2006). However, in our recordings, the firing durations (< 1 s for

200 Hz) within O-HFS were too short and the subsequent “deafferentation” durations were too long (see Table 1) to be explained by a synaptic failure such as transmitter depletion, since 50 Hz O-HFS could induce persistent population spikes for periods about ~1 min long. Therefore, there must have a failure of axons that prevented the HFS to reach the synapses and the post-synaptic cells, and resulting in “silent” cells with intact excitability.

Third, the strong evidence of the axon block mechanism is that the CA1 cell bodies can still fire orthodromically-evoked PS normally while they fail to respond an ongoing antidromic HFS. Only a block at the efferent axons can explain the phenomenon, because only cell body and axons are involved in the antidromic stimulation pathway, no synaptic transmission.

Block of axonal conduction by HFS has been observed in brain slices (Jensen and Durand 2007, 2009; Zheng et al., 2011) and in model simulations (Bellinger et al., 2008, Ratas and Pyragas, 2012). Based on our knowledge, there is only one publication suggesting that this phenomenon can also be reproduced in-vivo (Jensen and Durand, 2009). The results reported above were also obtained in rat hippocampal CA1 region in-vivo with biphasic stimulation pulses instead of monophasic pulses, and clearly indicate the presence of an axonal block mechanism underlying the long HFS. Moreover, the unique finding of this work is that the neuronal cell bodies are still able to fire while they fail to respond the ongoing HFS at afferent or efferent axons. These results indicate that HFS with biphasic pulses can generate a reversible and temporary functional disconnection in hippocampal axons in-vivo that can separate the input or output pathways from somata or their targets.

Plausible mechanisms of axonal conduction failure

There are several mechanisms that could explain the appearance of axonal block during HFS, including the depolarization blockade induced by high extracellular potassium concentration ($[K^+]_o$), desynchronization of action potentials and inhibition from interneuron network.

One plausible mechanism for the axonal block is high $[K^+]_o$ induced by HFS. Previous research has shown that repetitive stimulation can enhance $[K^+]_o$ to concentrations as high as 18 mM in the CA1 region (Haglund and Schwartzkroin, 1990). In addition, axonal block has been observed in rat spinal nerves by elevation of $[K^+]_o$ (Orchardson 1978). Simulation studies have also shown that high $[K^+]_o$ can block axonal conduction (Liu et al., 2009). Considering the small extracellular volume within axon fiber bundles and in the periaxonal space, the accumulated $[K^+]_o$ by HFS may not be able to diffuse out quickly as indicated by computer simulations (Bellinger, 2008). Following a quick suppression on the onset of HFS, the axonal conduction was almost maintained in a steady low level without decreasing further (see Fig.3). Presumably, the small supplement of $[K^+]_o$ by the suppressed action potentials of axons during prolonged HFS was balanced by the slow clearance of $[K^+]_o$. Therefore, as long as stimulation is applied with high frequency, the $[K^+]_o$ could be maintained at a high level sufficient to block conduction in most axon fibers (Jensen and Durand, 2009). In addition, the higher the stimulation frequency, the quicker the axons could be blocked, suggesting that $[K^+]_o$ was accumulating more rapidly. The result is consistent with those obtained with axonal loose patch clamp experiments showing that as the stimulation frequency increases (above 100 Hz), conduction fidelity decreases along with increasing potassium concentrations (Meeks et al., 2005). High $[K^+]_o$ could result in the suppression of axon conduction through a depolarization block mechanism (Bikson et al., 2001, 2003; Bellinger et al., 2008).

Another possible mechanism is desynchronization. Desynchronization of axonal firing could also generate a decreased amplitude of PS activity (Casey and Blick, 1969) while still

allowing neural firing. At the onset of HFS, each pulse induced PS activity with large amplitude. Quickly, axons were suppressed and then subgroups of axons fired action potential in turn. Every pulse of HFS could only activate a few of the axons, the next pulse activated other axons, thereby causing an apparent decrease of neural activity as showed in Fig. 3. However, this mechanism is unlikely based on data obtained in-vitro whereby the compound action potential width is not affected while its amplitude is decreased (Jensen and Durand, 2009).

Yet another mechanism is inhibition from interneuron network. Although HFS of axons could simultaneously activate the interneurons in both the feed-forward and feed-back inhibition networks (Alger and Nicoll, 1982; Leung et al., 2008), axonal activation and suppression does not involve synaptic transmission. Furthermore, repetitive stimulation may induce a use-dependent decrease in GABA-activated inhibitory postsynaptic potentials in rat hippocampus (McCarren and Alger, 1985; Pearce et al., 1995). The inhibitory activity could fade fast during HFS. Therefore, inhibitory synaptic activity of the GABAergic interneurons is unlikely a mechanism of axonal block during HFS.

Finally, although axonal block plays an important role in our results, other mechanisms, such as synaptic modification (See Osorio et al., 2005 for a review), could not be absolutely excluded since the axonal conduction was not totally eliminated (see Fig. 3). The remaining axonal inputs during a long O-HFS could also have effects on synapses and even on post-synaptic neurons. However, the data in present study have shown that axonal failure could be an essential mechanism of HFS.

Conclusion

The role played by axons in mediating the effects of DBS has recently attracted marked attention (Deniau et al., 2010; Zheng et al., 2011). Clinical investigations have shown that DBS in the axonal fiber tract in thalamic region can provide more effective therapy for the improvement in Parkinsonian symptoms (Lanotte et al., 2002; Yokoyama et al., 2001; Montgomery and Gale, 2008). The results of the present work suggest that axonal block could play an important part of the mechanisms involved in the therapeutic effect of DBS. For example, axonal block could control seizures by preventing the synchronization of neural activity through synaptic transmission as well as the propagation of epileptiform activity that requires axonal transmission. In a word, this functional disconnection in axons between upstream and downstream cells could have extensive implication for various other clinical applications of HFS in brain diseases.

4. Experimental Procedure

4.1 Surgical procedures

All procedures used in this study were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (China Ministry of Health). Adult Sprague Dawley rats (250 - 350 g) were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting Co.). The skull was partially opened to allow the placement of electrodes. A 16 channel electrode array (NeuroNexus Technologies) was inserted into the hippocampal CA1 region (AP, -3.5; ML, 2.7; DV 2.5) as recording probe. Two stimulation electrodes, concentric bipolar stainless-steel electrodes (FHC, Bowdoin, ME 04287, USA), were positioned respectively in the Schaffer collaterals (AP, -2.2; ML, 2.0; DV 2.8) for orthodromic stimulation and in the alveus (AP, -4.8; ML, 2.7; DV 2.3) for antidromic stimulation. Two separate stainless steel screws were fixed in the nose bone and served as reference electrode and ground electrode, respectively. Patterns of the evoked potentials were judged to guide the final positions of the recording probe and the stimulation electrodes. Normal saline was placed over the exposed surface of the dura to keep it moist.

4.2 Recording and Stimulation

Potential signals from recording probes were amplified by a 16-channel extracellular amplifier (Model 3600, A-M system Inc.) with a filtering frequency range of 0.3 Hz - 5 KHz. The amplified signals were then sampled by a ML880 Powerlab 16/30 data acquisition system (ADInstruments Inc.) at a sampling rate of 20 KHz/channel with 16-bit precision, and were stored into a hard disk for off-line analysis. The channel with largest evoked population spikes (PS) and dense unit activity was taken as the location in the pyramidal layer (Pyr.) of the CA1 region. Another channel located 400 μ m below the pyramidal layer channel was taken as the location of stratum radiatum (S. rad.) for the assessment of evoked fEPSP. Custom-made MATLAB codes were used to remove the stimulation artifacts within HFS in the recordings by blanking 1.2 ms signal at every stimulation time. The algorithm was able to remove the artifacts with minimal distortion of the neural signals (See Fig. 1B).

Biphasic HFS pulses with constant currents were generated by an isolated pulse stimulator (Model 2100, A-M system Inc.) with a pulse duration of 0.1 ms per phase. Input-output (I/O) curves of the orthodromic- or antidromic-stimulations were generated at a pulse frequency lower than 0.05 Hz with a current range of 0.1 - 0.6 mA. A current intensity (0.3 - 0.5 mA) that evoked PSs with an amplitude approximately 3/4 of the maximal PS amplitude was used as HFS stimulation current. The inter-pulse intervals within HFS trains were set according to stimulation frequencies of 50, 100 and 200 Hz, and the train lengths were set as 1 min. The intervals between sequential stimulation trains were greater than 30 min to ensure recovery from previous stimulation.

All statistical data were represented as mean $\pm$ standard deviation. One-way ANOVA with Post hoc Bonferroni test were used to judge the significance of the differences among data groups.

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Highlights

- Changes of evoked potentials during both orthodromic- and antidromic-HFS were studied.
- The CA1 somata remained excitable while failing to respond HFS of axons.
- HFS produces an axonal block of both afferent and efferent fibres.
- HFS causes a functional disconnection of axonal pathways that is reversible and temporary.

Fig. 1.

Neural activity during HFS. Small arrows over the waveforms indicate the stimulus timing. (A) Schematic diagram of experimental preparation showing the sites of the orthodromic- and antidromic-stimulation electrodes (OSE, ASE) in Schaffer collateral and alveus, respectively. The recording electrode array (RE) is inserted in the CA1 region. Typical baseline orthodromical- and antidromical-paired-pulses evoked PSs in the CA1 pyramidal layer. (B) Neural activity during a 1 min long period of 100 Hz O-HFS (top) and expanded insets with stimulation artifacts removed (middle) accompanied by those with stimulation artifacts (below). The artifact removal algorithm does not distort the signal. (C) Neural recording during a 1 min long period of 100 Hz A-HFS. In (B) and (C), the horizontal red bars over the signals indicate the stimulation duration. The time expanded insets show the changes of neural activity within different periods of the HFS.

Fig. 2.

Neural activity during 50 and 200 Hz HFS. Stimulation artifacts were removed to reveal the neural signals. The horizontal red bars over the signals indicate the stimulation duration. (A) Neural activity induced by 1 min long period of O-HFS at 50 and 200 Hz. 200Hz generated reduced PS activity. The extent of time with obvious PS activity (T_{ps}) is measured from the onset of O-HFS to the last PS within the HFS. (B) Neural activity induced by similar pulse trains as in (A) but with antidromic stimulation. 200Hz generated much reduced PS activity. Higher stimulation frequency generally evoked smaller PS amplitude. The antidromic PS amplitude ratio (R_{ps}) is defined as the percentage of the last stimulus evoked PS divided by the first stimulus evoked PS.

Fig. 3.

Changes of evoked PSs during a typical 1 min long period of 200 Hz A-HFS with a stimulation intensity of 0.5 mA. Each dot represents one stimulus-evoked PS. (A) Changes of APS amplitude (up) and latency (down) within the stimulation. The insets indicate the corresponding measurements with arrows pointing the artifacts. (B) Time expanded data of the first 1.0 s stimulation. The inset on the right shows the overlaid APS waveforms at 0, 0.25, 0.5, 0.75 and 1.0s that are aligned at the stimulating artifacts indicated by the arrow.

Fig. 4.

Changes of APS amplitudes in I/O curves compared to those during the onset of A-HFS. (A) A typical I/O curve of alveus stimulation showing the changes of APS amplitudes (up) and latencies (down) as a function of the stimulation intensity. The inset on the right shows the overlaid PS waveforms at 0.1 to 0.6 mA stimulation intensity. With the increase of stimulation intensity, the PS amplitudes increased significantly, but the PS latencies were left unchanged. (B) Comparison of the “Δ Amplitude” (up) and “Δ Latency” (down) between I/O and 50, 100, 200 Hz A-HFS evoked PSs. The data in “I/O” group taking at low frequency are the difference between the 0.1 and 0.6 mA stimulation evoked APSs in I/O curves; the data in the three “HFS” groups are the differences between the first and the 51th APSs in A-HFS. The test results of one-way ANOVA are shown on the right. * $P<0.02$ and ** $P<0.001$, Post hoc Bonferroni test.

Fig. 5.

Responses evoked by antidromic test stimuli (ATS) during O-HFS. (A) A typical signal recording obtained during a 1min long period of 200 Hz O-HFS with stimulation artifacts removed for clarity. The expanded insets show the ATS-evoked APS waveforms (shadings) with small arrows over the waveforms indicating the trimmed artifacts of 200 Hz O-HFS, and the yellow dots below waveforms indicating the artifacts of ATS. (B) Changes of normalized APS (up) and OPS (down) amplitudes for four 100 Hz and six 200 Hz 1 min long O-HFS with 0.2 Hz ATS ($n = 10$). Shadings with red bars on top indicate HFS durations. *Left*: PSs within and after HFS. *Right*: time expansion of PSs during HFS. APSs were evoked every 5 s within HFS and every 1 min after HFS. Please note, for comparison, during HFS, only samples of OPS were taken every 5 s (~20 ms before each ATS, with the first sample following the first stimulus of HFS). After the O-HFS, identical orthodromic-stimuli were applied every 1 min to show the recovery of OPS.

Fig. 6.

Responses evoked by orthodromic test stimuli (OTS) during A-HFS. (A) A typical signal recorded in the Pyr. and S. rad. during a 1 min long period of 100 Hz A-HFS with stimulation artifacts removed for clarity. The expanded insets below show the OTS-evoked fEPSP and OPS waveforms (shadings) with small arrows over the waveforms indicating the trimmed artifacts of 100 Hz A-HFS, and the yellow dots below waveforms indicating the artifacts of OTS. (B) Changes of normalized APS amplitudes (up), OPS amplitudes (middle) and fEPSP slope (bottom) for four 100 Hz and five 200 Hz 1 min long A-HFS with 0.2 Hz OTS ($n = 9$). Shadings with red bars on top indicate HFS durations. *Left*: Data within and after HFS. *Right*: time expansion of data during HFS. OPSs were evoked every 5 s within HFS and every 1 min after HFS. Please note, for comparison, during HFS, only samples of APS were taken every 5 s (~20 ms before each OTS, with the first sample following the first stimulus of HFS). Following A-HFS, identical antidromic-stimuli were applied every 1 min to show the recovery of APS.

Table 1
Comparison of PS activity induced by 1-min long O-HFS and A-HFS with different stimulation frequencies of 50, 100 and 200 Hz (mean $\pm$ standard deviation)

HFS mode	HFS frequency (Hz)	n	HFS intensity ^a (mA)	Amplitude of the first PS ^b (mV)	T _{PS} ^c (S)	R _{PS} ^d (%)
O-HFS	50	8	0.44 $\pm$ 0.13	8.20 $\pm$ 1.77	40.68 $\pm$ 20.84	-
	100	8	0.40 $\pm$ 0.09	8.58 $\pm$ 2.91	9.30 $\pm$ 12.76 *	-
	200	9	0.42 $\pm$ 0.08	8.38 $\pm$ 1.63	0.65 $\pm$ 1.08 *	-
A-HFS	50	9	0.46 $\pm$ 0.09	8.83 $\pm$ 1.90	-	36.47 $\pm$ 13.30
	100	10	0.46 $\pm$ 0.07	7.64 $\pm$ 2.36	-	16.21 $\pm$ 7.55 *
	200	9	0.44 $\pm$ 0.07	8.29 $\pm$ 2.99	-	6.07 $\pm$ 2.62 *

^{a,b} For both O-HFS and A-HFS mode, one-way ANOVA, $F < 0.6$, $P > 0.5$.

^c For O-HFS mode, one-way ANOVA, $F = 18.8$, $P < 0.001$.

^d For A-HFS mode, one-way ANOVA, $F = 27.3$, $P < 0.001$.

* Post hoc Bonferroni test $P < 0.001$ for comparisons 100 vs. 50 Hz, or 200 vs. 50 Hz.