Cathodic- and anodic-pulses can alternately activate different sub-populations of neurons during sustained high-frequency stimulation of axons in rat hippocampus

Zhouyan Feng, Lvpiao Zheng, Yue Yuan, Gangsheng Yang, Yifan Hu, Chuchu Lu and Zhaoxiang Wang

Key Laboratory of Biomedical Engineering for Ministry of Education, College of Biomedical Engineering and Instrument Science, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China

* Author to whom any correspondence should be addressed.
E-mail: fengzhouyan@zju.edu.cn

Keywords: neural stimulation, charge balance, anodic-pulse, biphasic-pulse, population spike, hippocampal CA1 region

Abstract

Objective. Charge-balanced biphasic-pulses are commonly used in neural stimulations to prevent possible damages caused by charge accumulations. The lagging anodic-phases of biphasic-pulses may decrease the activation efficiency of stimulations by counteracting the depolarization effect of the leading cathodic-phases. However, a monophasic anodic-pulse alone can itself activate neurons by depolarizing neuronal membrane through a mechanism of virtual cathode. This study aimed to verify the hypothesis that the anodic-phases/pulses in charge-balanced stimulations could play an activation role during sustained high-frequency stimulations (HFSs).

Approach. Two types of antidromic HFS (A-HFS) were applied on the alveus of hippocampal CA1 region of anesthetized rats: monophasic-pulse A-HFS of alternate opposite pulses and biphasic-pulse A-HFS with the same frequency of 100 or 200 Hz. The antidromically-evoked population spike was used as a biomarker to evaluate the activation effects of A-HFS pulses. Main results. Despite a significant difference in the initial abilities of anodic- and cathodic-pulses to activate neurons, an anodic-pulse was able to induce similar amount of neuronal firing as a cathodic-pulse during sustained monophasic-pulse A-HFS. Additionally, the amount of neuronal firing induced by the monophasic-pulse A-HFS was similar to that induced by the biphasic-pulse A-HFS consuming a double amount of electrical energy. Furthermore, the alternate cathodic- and anodic-pulses respectively activated different sub-populations of neurons during steady A-HFS. Significance. The anodic-phases/pulses in charge-balanced HFS at axons can play an activation role in addition to a role of charge balance. The study provides important information for designing charge-balanced stimulations and reveals new mechanisms of neural stimulations.

1. Introduction

Extracellular stimulations of electrical pulse sequences have been utilized in neural therapies such as deep brain stimulation (DBS) for treating certain brain disorders, as well as cochlear implants and intracortical visual stimulations for neural prostheses [1]. To minimize the risks of damages to both stimulated tissues and metal electrodes, charge-balanced biphasic pulses are commonly used [2–4]. The leading phase of the biphasic pulse is cathodic and acts as a working phase to activate neurons, while the lagging phase is anodic and acts as a balance phase to deliver opposite charges to neutralize the charges of the leading cathodic-phase, thereby preventing damages caused by possible charge accumulations [5–7].

The addition of anodic-phases cannot only double the consumption of electrical energy but also decrease the activation efficiency of stimulations by hyperpolarizing neuronal membranes to counteract the depolarization effect of the leading cathodic-phase [6, 8–10]. To improve the activation efficiency of biphasic pulses, asymmetric charge-balanced...
pulses have been utilized, such as a rectangular cathodic-phase followed by a smaller and longer anodic-phase of rectangular or non-rectangular waveform [2, 11, 12]. In addition, short inter-phase-gaps (IPGs) have been inserted between the cathodic- and anodic-phases to decrease the counteraction of anodic-phase [9, 13].

Nevertheless, a monophasic anodic-pulse itself can activate neurons by depolarizing neuronal membrane. For extracellular stimulations, due to the closed-loop flow of an electrical current, the anodic-pulse hyperpolarizing neuronal membrane in the vicinity of electrode must simultaneously depolarize membrane at distant sites on the same neuron [14, 15]. Especially for the long and thin axonal structure of neurons, an anodic-pulse can depolarize axonal membrane at flanking regions of the stimulation site and generate propagable action potentials (APs) [14, 16]. Although the activation efficiency of an anodic-pulse is much smaller than a cathodic-pulse under a normal situation [6, 15, 17], we proposed here that anodic-pulses/phases could play an important role in activating neurons with certain paradigms of stimulations, such as sustained high-frequency stimulations (HFSs) commonly used in DBS.

HFS sequences of biphasic-pulses around 100–200 Hz have been utilized in clinic DBS [18, 19]. In addition, axonal activations by HFS have been shown to play a crucial role in DBS therapy [20, 21], because axons occupy a large portion of brain space, and because an axon has a smaller rheobase current than other neuronal elements and is most prone to be activated by narrow pulses of HFS [14, 22–24]. However, sustained HFS may generate intermittent axonal blockage to decrease the evoked firing of axons/neurons because of an excessive depolarization of axonal membranes by HFS [25–27]. Under this situation, an addition of a reverse effect by anodic-pulses could be expected to alleviate the axonal blockage and to increase the activation effect of HFS on axons.

Based on the considerations above, we hypothesized that by separating two phases of biphasic-pulses with an IPG to form a HFS sequence composed of alternate monophasic cathodic- and anodic-pulses in axonal stimulations, the anodic-pulses could activate neurons themselves rather than only balance charges. To test the hypothesis, we utilized the antidromically-evoked population spike (APS) in rat hippocampus as a biomarker to evaluate the activation effects of different types of pulses in HFS sequences. The results may reveal new effects of anodic-pulses/phases in charge-balanced HFS and provide important information for developing stimulation paradigms to improve activation efficiency and to save electrical energy in neural stimulation therapies.

2. Materials and methods

2.1. Animal surgery and electrode implantations

Experiments were performed on adult male Sprague-Dawley rats in a stereotaxic apparatus under anesthesia by urethane (1.25 g kg$^{-1}$, i.p.). Surgical procedures and electrode implantations were similar to previous reports [26]. Briefly, a recording electrode (RE) and a stimulation electrode (SE) were inserted into the hippocampal CA1 region of left brain with coordinates in millimeter (AP $\sim$3.5; ML 2.7; DV 2.3) and (AP $\sim$4.8; ML 2.7; DV 2.3), respectively. The distance between the RE and SE was $\sim$1.3 mm (figure 1(A)). The RE was a 16-channel array (IPolytrode, A1x16-Poly2-5 mm-50 s-177, Neuro-Nexus Technologies Inc., USA) and was positioned perpendicularly across the layers of CA1 region activated by stimulations from SE. The SE was a bipolar concentric electrode (#CBCS75, FHC Inc., USA) and was targeted at the axons of CA1 pyramidal neurons, the alveus fiber of CA1 region (figure 1(A)). The diameters of inner and outer poles of the bipolar SE were 75 and 250 µm respectively, and the heights of both poles were 100 µm with a separation distance of 100 µm.

The spontaneous unit spikes as well as the typical waveforms of evoked potentials (including APS and because an axon has a smaller rheobase current than other neuronal elements and is most prone to be activated by narrow pulses of HFS [14, 22–24]. However, sustained HFS may generate intermittent axonal blockage to decrease the evoked firing of axons/neurons because of an excessive depolarization of axonal membranes by HFS [25–27]. Under this situation, an addition of a reverse effect by anodic-pulses could be expected to alleviate the axonal blockage and to increase the activation effect of HFS on axons.

Based on the considerations above, we hypothesized that by separating two phases of biphasic-pulses with an IPG to form a HFS sequence composed of alternate monophasic cathodic- and anodic-pulses in axonal stimulations, the anodic-pulses could activate neurons themselves rather than only balance charges. To test the hypothesis, we utilized the antidromically-evoked population spike (APS) in rat hippocampus as a biomarker to evaluate the activation effects of different types of pulses in HFS sequences. The results may reveal new effects of anodic-pulses/phases in charge-balanced HFS and provide important information for developing stimulation paradigms to improve activation efficiency and to save electrical energy in neural stimulation therapies.

2.2. Recording and stimulating

Electrical potentials collected by the RE were amplified 100 times by a 16-channel extracellular amplifier (Model 3600, A-M System Inc., USA) with a band-pass filtering range of 0.3–5000 Hz. Then the amplified signals were sampled by a PowerLab data acquisition system (Model PL3516, ADInstruments Inc., Australia) with a sampling rate of 20 kHz per channel.

Stimulation pulses applied on the alveus were monophasic cathodic- and anodic-pulses, denoted as $\top$ and $\bot$ respectively, and symmetric biphasic-pulses with cathodic-phase first and zero IPG, denoted as $\spadesuit$. The stimuli were all rectangular current pulses with a width of 100 µs per phase and a current intensity of 0.3–0.4 mA per phase, and were generated by a programmable stimulator (Model 3800, A-M System...
Figure 1. Neuronal responses to A-HFS of alternate cathodic (⊤) and anodic (⊥) pulses at 100 and 200 Hz in the rat hippocampal CA1 region. (A) Schematic diagram of the locations of the RE in the CA1 region and the SE in the alveus, together with APS waveforms evoked by a single ⊤ and ⊥ pulse (left). A histological picture of sagittal slice shows the trace of SE to the alveus, indicated by the line of bloodstain through cortex to the dorsal hippocampus (right). (B) A typical recording of neuronal responses to a 2 min sequence of 100 Hz A-HFS of alternate ⊤ and ⊥ pulses. The expanded insets show examples of APS waveforms following the two types of pulses at different periods of A-HFS. Dot lines denote removed stimulation artifacts. (C) For 100 Hz A-HFS (n = 15), typical scatter plot of APS amplitudes evoked by each pulse (C1), comparisons of the initial APS amplitudes (A⊤ini vs A⊥ini, (C2)) and the steady APS amplitudes (A⊤end vs A⊥end, (C3)), and comparisons of the initial APS latencies (L⊤ini vs L⊥ini, (C4)), and the steady APS latencies (L⊤end vs L⊥end, (C5)). The A⊤end, A⊥end, L⊤end and L⊥end were the average values in the last 1 s of A-HFS. (D) For 200 Hz A-HFS (n = 13), comparisons of the initial (D1) and steady (D2) APS amplitudes as well as the initial (D3) and steady (D4) APS latencies. *P < 0.05, **P < 0.01, paired t-test, n = 15 or 13.
Inc., USA). The current intensity of the pulses was able to evoke APSs with an amplitude approximately 3/4 of the maximal amplitude in the input–output curve of APS amplitudes.

Two types of antidromic HFS (A-HFS) were applied on the alveus: biphasic-pulse A-HFS and monophasic-pulse A-HFS of alternate cathodic- and anodic-pulses. Both types of A-HFS were charge-balanced sequences. The polarity of pulses was defined according to the polarity of current flowing through the inner pole of the bipolar SE. The pulse frequency of A-HFS was 100 or 200 Hz. The frequency of monophasic-pulse A-HFS was defined as the sum number of cathodic-pulses and anodic-pulses per second. The duration of A-HFS was 2 min.

2.3. Data analysis

The amplitude and latency of evoked APS waveforms were used to evaluate the neuronal responses to different types of pulses. The APS amplitude was measured as the potential drop of the negative peak of APS. The APS latency was the time distance between the stimulation pulse and the negative peak of APS. The evoked APSs were steady in the second minute of the 2 min A-HFS. An average value of APS waveforms calculated by the data in the last 1 s of A-HFS (denoted by a subscript ‘end’) was termed as a steady value to describe the evoked APSs during the steady period. To clarify illustrations, stimulation artifacts in the A-HFS recordings were removed by a custom-made MATLAB program as previous reports [29]. All statistical data were represented as mean ± standard deviation with n representing the number of rats for data collections. Paired t-test, one-way analysis of variance (ANOVA) or two-way ANOVA with post-hoc Bonferroni tests were used to judge the statistical significances of the differences among data groups.

3. Results

3.1. Responses of neuronal populations to monophasic-pulse A-HFS with alternate cathodic- and anodic-pulses

A single pulse applied at the alveus can activate the axon fiber and then antidromically evoke APs in the cell bodies of pyramidal neurons in the hippocampal CA1 region. The synchronous firing of the neuronal population forms an APS waveform that can be recorded extracellularly in the pyramidal cell layer to evaluate the stimulation effect [30]. With an identical current intensity, a pulse always evoked a larger APS than a pulse (figure 1(A, left)).

At the onset of a 100 Hz monophasic-pulse A-HFS (i.e. 50 Hz for and pulses respectively), the two types of pulses alternately evoked larger and smaller APSs (figure 1(B, bottom left)), similar to single pulse stimulations at baseline. As the A-HFS continued, the APS amplitudes decreased rapidly, indicating failures of neurons to follow each of the high-frequency pulses. Interestingly, the APSs evoked by pulses decreased first to almost disappear and then returned to a steady level, while the APSs evoked by pulses decreased monotonically to a steady level. After ~40 s stimulation, the amplitudes of APSs evoked by both types of pulses became similar (figures 1(B) and (C1)). In the experiments with 100 Hz A-HFS, the amplitudes of initial APS evoked by the first and pulses ( and ) were significantly different (figure 1(C2); P < 0.01, paired t-test, n = 15). The mean was only about half of the mean . However, the amplitudes of steady APSs evoked by the two types of pulses were similar ( and in figure 1(C3)). The difference between the amplitudes of the two types of APSs significantly decreased from the initial value ( ) to the steady value ( ) (figure 1(C4)). The significant differences between the mean latencies of the two types of APSs existed through the entire period of A-HFS ( ) vs ( ) in figure 1(C5) and ( ) vs ( ) in figure 1(C6); P < 0.01, paired t-test, n = 15). Furthermore, the latencies of both types of APSs nearly doubled from their initial values to steady values, resulting in the difference of the two latencies significantly increasing from the initial value ( ) to the steady value ( ) (figure 1(C7)). The mean latency of both types of APSs was significantly longer than that of the 100 Hz A-HFS (figure 1(C8)).

In addition, during the A-HFS, the latencies of all APSs gradually increased with the mean latency following pulses shorter than that following pulses (figure 1(C4)). The significant differences between the mean latencies of the two types of APSs existed through the entire period of A-HFS ( ) vs ( ) in figure 1(C5) and ( ) vs ( ) in figure 1(C6); P < 0.01, paired t-test, n = 15). Furthermore, the latencies of both types of APSs nearly doubled from their initial values to steady values, resulting in the difference of the two latencies significantly increasing from the initial value ( ) to the steady value ( ) (figure 1(C7)). The mean latency of both types of APSs was significantly longer than that of the 100 Hz A-HFS (figure 1(C8)).

During A-HFS with an increased pulse frequency of 200 Hz, the change of APS amplitudes was similar to the 100 Hz A-HFS (figures 1(D1) and (D2)). The difference between the amplitudes of the two types of APSs significantly decreased from the initial value ( ) to the steady value ( ) (figure 1(D3)) and ( ) in figure 1(D6); P < 0.01, paired t-test, n = 13). The APS amplitudes of pulses decreased to below 10% of the initial value and that of pulses decreased to below 20% (see figures 1(D1) and (D2)), approximately half of the ratios with 100 Hz A-HFS. Again, the mean latency of APSs evoked by pulses was significantly shorter than that evoked by pulses (figures 1(D3) and (D4); P < 0.05 or 0.01, paired t-test, n = 13), with the mean latency difference increasing significantly from the initial value ( ) to the steady value.
\(\Delta L_{\text{end}} = 0.23 \pm 0.15 \text{ ms, } P < 0.05, \text{ paired } t\text{-test, } n = 13\).

These results indicated that despite the significant difference in their initial activations on neurons, the \(\perp\) pulses were able to activate neurons as efficiently as \(\top\) pulses during sustained A-HFS of alternate pulses when the evoked APSs were suppressed. Previous studies have also shown suppressions of APS during biphasic-pulse A-HFS [26, 31]. We next compared the APSs evoked by the two types of A-HFS (monophasic vs biphasic) with a same pulse frequency and a same intensity, but with a different consumption of electrical energy.

3.2. Comparisons of APSs evoked by monophasic-pulse A-HFS and by biphasic-pulse A-HFS

At the initial period of a 100 Hz biphasic-pulse A-HFS, large APS (~10 mV) followed each pulse and then decreased rapidly in seconds (figure 2(A)). After tens of seconds of continuous stimulation, the APS amplitudes decreased to a steady level that was similar to the steady level of monophasic-pulse A-HFS (figure 2(B, left)). Meanwhile, the latencies of APSs evoked by biphasic-pulses increased to a steady level between the latencies of APS evoked by the two opposite pulses of monophasic-pulse A-HFS (figure 2(B, right)).

Statistical data showed that significant differences existed among the amplitudes of initial APSs of biphasic-pulses \(A_{L_{\text{ini}}}\) and of monophasic-pulses \(A_{L_{\text{ini}}}\) and \(A_{L_{\text{ini}}}\). Both \(A_{L_{\text{ini}}}\) and \(A_{L_{\text{ini}}}\) were significantly greater than \(A_{L_{\text{ini}}}\) (figure 2(C1)), \(**P < 0.01, \text{ two-way ANOVA with post-hoc Bonferroni tests, } n = 8\). During the steady period, the significant differences disappeared (figure 2(C2)). In addition, significant differences existed among the latencies of APSs evoked by the three types of pulses in both initial and steady periods of A-HFS. The latencies of APSs evoked by biphasic-pulses \(L_{\text{ini}}\) and \(L_{\text{end}}\) were significantly longer than those evoked by anodic-pulses \(L_{\text{ini}}\) and \(L_{\text{end}}\) and were significantly shorter than those evoked by cathodic-pulses \(L_{\text{ini}}\) and \(L_{\text{end}}\) (figures 2(C3) and (C4), \(**P < 0.01 \text{ or } *P < 0.05, \text{ two-way ANOVA with post-hoc Bonferroni tests, } n = 8\)). Similar results were obtained by comparing the amplitudes and latencies of APSs of the three types of pulses during A-HFS with a higher frequency of 200 Hz, except that the latency \(L_{\text{end}}\) was significantly shorter than both latencies \(L_{\text{end}}\) and \(L_{\text{end}}\) (figure 2(D)).

To compare the mean amounts of neuronal firing evoked by monophasic-pulse A-HFS and biphasic-pulse A-HFS, the amplitudes of APSs evoked by cathodic- and anodic-pulses in monophasic A-HFS were averaged (the rightmost bar with two colors in figures 2(C1), (C2), (D1) and (D2)). For 100 Hz A-HFS, at the initial period, the average amplitude \((A_{L_{\text{ini}}} + A_{L_{\text{ini}}})/2\) of monophasic-pulses was significantly smaller than the amplitude \(A_{L_{\text{ini}}}\) of biphasic-pulses (figure 2(C1), \(P < 0.05, \text{ paired } t\text{-test, } n = 8\). At the steady period, the \((A_{L_{\text{end}}} + A_{L_{\text{end}}})/2\) became similar to \(A_{L_{\text{end}}}\) (figure 2(C2)), indicating similar neuronal firing evoked by A-HFS of alternate monophasic- and biphasic-pulses. Similar results of APS amplitudes were obtained during A-HFS with a higher frequency of 200 Hz (figures 2(D1) and (D2)).

In addition, to compare the recoveries of neuronal activity after the two types of A-HFS, single test pulses with the identical parameters as A-HFS pulses were repeatedly applied with an interval of \(\sim 30\) s after the end of A-HFS. For the both types of A-HFS, over 90% changes in the APS amplitudes and latencies recovered in \(\sim 2\) min following the end of A-HFS, indicating no obvious neuronal damages caused by these A-HFS (figure 3).

These results showed that although significant differences existed in the initial APSs, the alternate monophasic-pulse A-HFS and the biphasic-pulse A-HFS suppressed APSs to a similar level during the steady period of A-HFS. The suppressed APSs indicated that each of the pulses only activated a small fraction of the neuronal population that was covered by the pulses in baseline situation or at the onset of A-HFS. Because the activation sites of the \(\top\) and \(\perp\) pulses along an axon should be different [7, 14, 32], we hypothesized that the suppressed APSs evoked by the \(\top\) and \(\perp\) pulses of monophasic-pulse A-HFS could be formed by the firing from different sub-populations of neurons, which was different from biphasic-pulse A-HFS. The hypothesis was verified next.

3.3. Cathodic- and anodic-pulses activate different sub-populations of neurons during steady period of monophasic-pulse A-HFS

We utilized the theory of refractory period following neuronal firing to test whether or not two consecutive pulses activate a same population of neurons [33, 34].

When pairs of biphasic-pulses with identical parameters and with a short inter-pulse interval (IPI) were applied in baseline situation, the second pulse (termed as test pulse) only induced a small APS with an IPI of 1.5 ms following the first pulse (termed as control pulse), and induced no APS with an IPI of 0.8 ms (figure 4(A)). The result indicated a refractory period of \(\sim 1\) ms under the baseline situation. However, during sustained 100 Hz A-HFS of the biphasic-pulses with the identical parameters, an additional pulse, even inserted 5 ms following a pulse of A-HFS, induced no APS (figure 4(B)), indicating a refractory period longer than 5 ms at this time. The result was consistent with previous report that the refractory period of neuronal firing may be extended by A-HFS [31].

As a comparison, pairs of monophasic-pulses were applied with a leading \(\top\) pulse followed by a \(\perp\) pulse in baseline situation. The \(\perp\) pulse only induced
Figure 2. Comparisons of the amplitudes and latencies of evoked APS between A-HFS of alternate monophasic-pulses and A-HFS of biphasic-pulses. (A) A typical recording of neuronal responses to a 2 min 100 Hz A-HFS of biphasic-pulses (┼). The expanded insets show examples of APS waveforms at different A-HFS periods. Dot lines denote removed stimulation artifacts. (B) Scatter plots of the amplitudes (left) and latencies (right) of APS evoked by each biphasic-pulses (in green) during the A-HFS shown in (A), together with the corresponding data of APS evoked by the cathodic (⊤) and anodic (⊥) pulses (in blue and orange respectively) during an A-HFS of alternate monophasic-pulses in the same rat experiment. (C) Comparisons of the initial and steady values of APS amplitudes ((C1) and (C2)) and latencies ((C3) and (C4)) among APSs evoked by monophasic ⊤ pulse, ⊥ pulse and biphasic-pulses for 100 Hz A-HFS. (C1) and (C2) also include the comparisons between the values of biphasic-pulses and the average value of ⊤ and ⊥ pulses of monophasic-pulses (the rightmost bar with two colors). (D) Corresponding comparisons for 200 Hz A-HFS. *P < 0.05, **P < 0.01, two-way ANOVA (factors: pulse type and individual rat) with post-hoc Bonferroni tests, n = 8. *P < 0.05, **P < 0.01, paired t-test, n = 8.
Figure 3. Recovery of neuronal responses to pulse stimulations following A-HFS of alternate monophasic-pulses and A-HFS of bi-phasic. (A) Typical recordings of neuronal responses to single pulse tests before and after the two types of A-HFS. The time axis at the bottom denotes the timings of test pulses and the A-HFS period. The insets show examples of APS waveforms evoked by test pulses at different times. (B), (C) Changes of the mean APS amplitude normalized by baseline values for the two types of A-HFS. The shade with a bar on top denotes the A-HFS period. During the A-HFS periods, the first data dot was from the initial single APS, while the other data dots were the average of APS values in the last 1 s for every 5 s. The data dots following A-HFS were from single pulse tests in an interval of 30 s. (D) Comparisons of the mean times for the APS amplitudes to recover to 90% of baseline value ($T_{90\%}$) calculated by a linear interpolation between two adjacent data shown in (B) and (C). (E)–(G) Latency data corresponding to the APS data shown in (B)–(D). The recovery time of APS latency was evaluated by the time when the increased latency fell to 110% of baseline value ($T_{110\%}$). The error bars in (B)–(G) are all one standard deviation.

A small APS with an IPI of 1.5 ms following the $\top$ pulse, and induced no APS with an IPI of 0.8 ms due to refractory period (figure 4(C)). This result suggested that the population of neurons activated by a $\bot$ pulse was included completely in the population of neurons activated by a $\top$ pulse in baseline situation.

During sustained 100 Hz A-HFS of alternate $\top$ and $\bot$ pulses, an additional pulse of $\top$ or $\bot$ inserted 5 ms following the two types of pulse generated different APSs (figure 4(D)). When the polarity of inserted pulse was same as the preceding A-HFS pulse, the inserted pulse induced no APS (figures 4(D1) and (D2)), similar to the results in inserting biphasic-pulse into A-HFS of biphasic-pulses (figure 4(E)). However, when the polarity of inserted pulse was opposite to the preceding pulse, it induced a substantial APS (figures 4(D3) and (D4)) with a mean amplitude of ~60%-70% of the APS induced by an immediately previous pulse with the same polarity (figure 4(E)). In addition, the inserted pulse resulted in no APS following the subsequent pulse with the same polarity (denoted by the hollow triangles in figures 4(D3) and (D4)). This again indicated the
Figure 4. Different activation patterns of alternate monophasic-pulses and biphasic-pulses during sustained A-HFS. (A) In baseline, examples of the control APS ($A_{\perp \text{control}}$) evoked by a single biphasic-pulse (left) and the APSs evoked by paired biphasic-pulses with IPI of 1.5 and 0.8 ms (right). The test APS ($A_{\perp \text{test}}$) evoked by the second pulse in pairs was suppressed due to refractory period. (B) A typical example of 2 min 100 Hz A-HFS of biphasic-pulses with test pulses inserted at $\sim 105$ and $\sim 115$ s in the middle of 10 ms IPI. No APS ($A_{\perp \text{ins}} = 0$) was evoked by the inserted pulses due to a putative extended refractory period. (C) In baseline, examples of the control APS ($A_{\perp \text{control}}$) evoked by a single $\perp$ pulse and the APSs evoked by paired $\|$ and $\perp$ pulses with IPI of 1.5 and 0.8 ms. The test APS ($A_{\perp \text{test}}$) evoked by the $\perp$ pulse was suppressed due to refractory period. (D) A typical example of 2 min 100 Hz A-HFS of alternate monophasic-pulses with test-pulses inserted at $\sim 100$, $\sim 105$, $\sim 110$ and $\sim 115$ s in the middle of 10 ms IPI. The inserted pulses formed four types of tests (orange boxes in (D1)–(D4)): insert $\|$ after $\|$ and insert $\perp$ after $\perp$. The hollow triangle in (D3) and (D4) denotes no APS induced by the pulse immediately following the inserted pulse. (E) The amplitude ratios of the APS evoked by the inserted test-pulse to the APS evoked by the preceding pulse with the same polarity as the test-pulse. In (A)–(D), the dot lines denote the removed stimulation artifacts of different types of pulses indicated by different colors: green for $\|$ pulses, blue for $\|$ pulses and brown for $\perp$ pulses, respectively.

effect of an extended refractory period following the APS induced by the inserted pulse.

These results suggested that the biphasic-pulses controlled a same population of neurons either in baseline or during sustained A-HFS of biphasic-pulses. However, the monophasic-pulses of opposite polarities controlled different sub-populations of neurons during sustained A-HFS of alternate monophasic-pulses, although in baseline a $\perp$ pulse only controlled a fraction of neurons that was covered by a $\|$ pulse.

4. Discussion

The novel findings of this study include: (a) an anodic-pulse can induce similar amount of neuronal
firing as a cathodic-pulse during sustained A-HFS of alternate monophasic-pulses, despite a significant difference in their initial abilities of neuronal activations. (b) The cathodic- and anodic-pulses can activate different sub-populations of neurons, respectively. (c) The monophasic-pulse A-HFS can induce similar amount of neuronal firing as the biphasic-pulse A-HFS that consumes a double amount of electrical energy. Possible mechanisms and implications underlying these results are discussed below.

4.1. Possible underlying mechanisms of axonal HFS with alternate cathodic- and anodic-pulses

It is interesting that an anodic-pulse can have an activation ability equivalent to that of a cathodic-pulse during sustained A-HFS. Generally, the depolarization effect of an anodic-pulse is much weaker than that of a cathodic-pulse with the same stimulation intensity, because an anodic-pulse depolarizes axonal membrane at the flanking regions of stimulation site through a mechanism of virtual cathode (figure 5(A)). Therefore, in the baseline situation (or at the initial of monophasic-pulse A-HFS), an anodic-pulse can only activate the axons in a smaller zone close to the SE with a smaller magnitude of depolarization at the flanking regions (indicated by zone1 in figures 5(A1) and (A2)), while a cathodic-pulse can activate both the close and distant axons with a greater magnitude of depolarization at the center region immediately under the stimulation site (indicated by zone1 and zone2 in figures 5(A1) and (A3)). This was confirmed by the significant smaller APS evoked by an anodic-pulse than that by a cathodic-pulse in the initial of monophasic-pulse A-HFS (figures 1(C2) and (D1)). In addition, a cathodic-pulse can generate hyperpolarization in the flanking regions that may hinder the propagation of AP initiated at the center region [32], thereby causing a longer latency of APS than that of APS induced by an anodic-pulse (figures 1(C5) and (D3)).

However, during sustained 100 and 200 Hz A-HFS, similar small APSs were evoked by the both types of pulses (figure 1). The suppression of APS is due to depolarization blockage induced in axons as reported previously with A-HFS [25, 26, 35, 36], which may be caused by an increase of extracellular potassium (\([K^+]_o\)) in the peri-axonal space by axonal HFS [27, 37, 38]. Nevertheless, an intriguing thing is that the suppression ratio of APS by anodic-pulses was smaller than that by cathodic-pulses (figure 3(B)), thereby resulting in the similar small APSs evoked by the both types of pulses. This could be due to the fact that the anodic-pulses generated weaker depolarizations and less accumulations
of \([K^+]_o\), thereby resulting in a weaker depolarization blockage and a smaller suppression ratio of APS.

Presumably, during steady period of A-HFS, the cathodic-pulses may no longer activate the axons in the zone closer to the electrode because of a stronger depolarization blockage generated by the pulses, but the anodic-pulses can. Thus, the anodic-pulses and the cathodic-pulses may activate the close zone and distant zone, respectively (indicated by zone1 and zone2 in figure 5(B1)). The experiment data of refractory period tests support this inference (figure 4). The two zones could overlap without a definite boundary line indicated by the black dot line in the figure 5(B1). Furthermore, each of the pulses, either anodic- or cathodic-pulses, may only activate a fraction of the axons in zone1 or zone2 (indicated by the radial dot lines in the zones in figure 5(B1)), thereby resulting in suppressed APSs. That is, the axons could only intermittently follow some of the pulses, not every pulse, to fire APs (figures 5(B2) and (B3)) due to a possible mechanism of \([K^+]_o\) accumulation [27, 38]. In addition, the difference in APS latencies evoked by the two types of pulses persisted through the entire A-HFS due to the differences in initial sites of APs and in the obstruction of hyperpolarization by cathodic-pulses. And, the latencies were all prolonged by the depolarization caused by \([K^+]_o\) accumulation during sustained A-HFS.

In the initial seconds of A-HFS, with the activation efficiency of pulses decreasing, the anodic- and cathodic-pulses may compete to activate axons. Due to its stronger ability of depolarization, the cathodic-pulses may first obtain a dominant position to prevent the anodic-pulses to activate axons and result in a sharp decrease of anodic-evoked APS even to a transient disappearance (figure 1(C1)). Then, after the depolarization blockages cause cathodic-pulses fail to activate the axons adjacent to the stimulation site, the anodic-pulses may obtain the opportunity to activate some axons through ‘virtual cathode’ at the flanks of stimulation site, and the anodic-evoked APSs reappear.

Furthermore, the distance between the SE and the target axons is one of the crucial factors determining the activation ability of delivered pulses [39–41]. In this study, the bipolar SE was positioned with its inner pole at the alveus (figure 1(A)). With a pulse intensity of 0.3 or 0.4 mA, the APS evoked by a separate cathodic-pulse was about twice as large as the APS evoked by a separate anodic-pulse in baseline, then both types of APSs became similar during the steady period of monophasic-pulse A-HFS (figure 1). If the electrode was a little far away from the alveus, an anodic-pulse would hardly activate the axons while a cathodic-pulse would be able to activate a small number of the axons to generate a small APS. In this case, during sustained A-HFS, the anodic-pulses would still not activate the axons to generate APS and only cathodic-pulses would induce suppressed APSs (data not shown).

It is impossible that the differences in the APSs evoked by anodic- and cathodic-pulses were caused by an activation of different elements of neurons. The stimulation was impossible to directly activate the somata around the RE that was ∼1.3 mm from the stimulation site (figure 1(A)). Also, the evoked potentials (APS) were impossible to be induced by synaptic inputs on dendrites since the APS latencies were all smaller than 2 ms at the initial period of A-HFS (figures 1(C5), (D3) and 2(C3), (D3)). In addition, the typical waveforms of evoked potentials serially appearing in the 16-channel RE ensured that the APSs were induced antidromically by activations of neuronal axons [42].

Another interesting finding of the present study is that with 100 and 200 Hz A-HFS, the amount of neuronal firing induced by the monophasic-pulse A-HFS was similar to that induced by the biphasic-pulse A-HFS (figure 2). Presumably, during sustained A-HFS with uniform biphasic-pulses, without the competition of separate anodic-pulses, the neurons in the close zone (zone1 in figure 5(B1)) can be either activated intermittently by the biphasic-pulses or totally blocked. In addition, the neurons in the distant zone (zone2 in figure 5(B1)) can be also activated intermittently by the biphasic-pulses but with a smaller firing amount per pulse than that induced by cathodic-pulses during monophasic-pulse A-HFS, because the double frequency of cathodic-phases in the biphasic-pulse A-HFS may generate a stronger depolarization blockage than monophasic-pulse A-HFS. Thus, the sum firing in the entire zone (zone1 plus zone2) evoked by the biphasic-pulses is similar to that evoked by monophasic-pulses, despite the fact that the electrical energy consumed by biphasic-pulses is twice as monophasic-pulses.

Taken together, with a proper position of SE at target axons, due to the putative mechanism of intermittent depolarization blockage of axons and the different initial sites of APs induced by the two types of pulses, the anodic-pulses can play an activation role similar to the cathodic-pulses in sustained monophasic-pulse A-HFS of alternate polarities. Nevertheless, the putative mechanisms need to be verified by more direct evidence in future studies with development of experimental technology.

4.2. Implications of HFS with alternate phases

Our present study firstly showed that sustained HFS can change the relationship of activation efficiencies between cathodic-pulses and anodic-pulses, which provides new information for designing charge-balanced stimulations for neural stimulations. The monophasic-pulse A-HFS used here (100 or 200 Hz) is a type of charge-balanced stimulation, equivalent to a sequence of biphasic-pulses (50 or 100 Hz)
with an IPG (10 or 5 ms). Previous studies have shown that an IPG of a fraction of millisecond to several milliseconds can reduce the reverse effect of the anodic-phase on the leading cathodic-phase in charge-balanced stimulations \[9, 10, 43\], so did the IPG of 5 or 10 ms used in this study. In a further step, here we showed that the anodic-phase/pulse itself can also play an activation role to induce neuronal firing similar as the cathodic-phase/pulse. It means that a waveform design of the anodic-phase other than rectangular pulse may not be necessary. Utilizing the activation effect of anodic-pulses in continuous stimulations can save the electrical energy of implanted stimulators. Moreover, the quick recovery of neuronal responses following the A-HFS indicated that the monophasic-pulse A-HFS at 100–200 Hz would not damage brain tissues (figure 3). This type of HFS was as safe as biphasic-pulse HFS.

Furthermore, the activation effect of monophasic-pulse HFS is different from that of biphasic-pulse HFS since our data showed that the opposite pulses of monophasic-pulse A-HFS can activate different sub-populations of neurons to fire alternately (figure 4). It provides a new stimulation pattern with certain selective activations of neurons. The alternate activations of sub-populations of axons may activate the post-synaptic neurons in a distinct way that is worthy of further investigations.

In addition, the present study provides new evidence to support the point that axonal HFS of 100–200 Hz can generate failures (such as depolarization blockage) at axons to prevent them to respond every pulse of HFS successfully \[25, 26, 35, 36\]. Otherwise, if the axons had been able to follow every pulse of the axonal HFS reliably to generate and conduct APs, the suppression of APS would have been caused by failures at somata. Under this situation, the somata of the neurons would have received similar antidromic inputs of the APs initiated by whatever cathodic- and anodic-pulses at axons, losing a marker of the pulse types. Therefore, the fact that different sub-populations were activated separately by the two types of pulses can only be due to failures at the axons immediately under HFS.

Nevertheless, the results of HFS of alternate monophasic-pulses observed in the present study were only from the rat hippocampal CA1 region. Further studies are needed to verify their universality in other brain regions as well as in spinal nerve, auditory nerve and so on. Moreover, the applicability of the type of HFS with alternate monophasic-pulses in neural stimulations for treating neural disorders needs further investigations of animal studies and clinical verifications.

5. Conclusions

The novel finding of the study is that sustained high-frequency axonal stimulations can enable an anodic-pulse/phase to activate neurons parallel to a cathodic-pulse/phase, rather than to only act as a balance phase. The finding not only provides new information for developing efficient and power-saving stimulations but also reveals new mechanisms of neural stimulations.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Acknowledgments

We thank Hanhan Hu and Weijian Ma for their help in animal experiments and data analyses. Special thanks to Xuefeng Wei (The College of New Jersey, USA) for his suggestions of possible underlying mechanisms and explanations.

Author contributions

Z F and L Z conceived and designed the study. L Z, Y Y, G Y, Y H, C L and Z W performed the animal experiments and analyzed the experimental data. Z F and L Z interpreted the results and wrote the manuscript. All authors approved the final version for submission.

Conflict of interest

The authors declare no competing interests.

Ethical statement

The animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (China Ministry of Health), and were approved by the Institutional Animal Care and Ethics Committee, Zhejiang University (Ethics Code: ZJU20210108).

Funding

This work was supported by the National Natural Science Foundation of China (No. 30970753).

ORCID iDs

Zhouyan Feng https://orcid.org/0000-0003-4110-4239
Lvpiao Zheng https://orcid.org/0000-0003-0478-7034

References

[1] Thompson D M, Koppes A N, Hardy J G and Schmidt C E 2014 Electrical stimuli in the central nervous system microenvironment Annu. Rev. Biomed. Eng. 16 397–430
[2] Montgomery E B 2017 Deep Brain Stimulation Programming: Principles, Techniques and Side Effects 2nd edn (Oxford: Oxford University Press)

[3] Wilson B S and Dorman M 2009 The design of cochlear implants Cochlear Implants: Principles and Practices 2nd edn, ed J K Niparko, K I Kirk, A Robbins, N K Mellon, D L Tucci and B S Wilson (Philadelphia: Wolters Kluwer Health) pp 95–135

[4] Shepherd B K, Shidlovski M N, Nayagam D A, Williams C E and Blamey P J 2013 Visual prostheses for the blind Trends Biotechnol. 31 562–71

[5] Lilly J C, Hughes J R, Alvord E C and Galkin T W 1955 Brief, noninjurious electric waveform for stimulation of the brain Science 121 468–9

[6] Merrill D R, Bilson M and Jefferys J G 2005 Electrical stimulation of excitable tissue: design of efficacious and safe protocols J. Neurosci. Methods 141 171–98

[7] Brocker D T and Grill W M 2013 Principles of electrical stimulation of neural tissue Handb. Clin. Neurol. 116 3–18

[8] Reilly J P, Freeman V T and Larkin W D 1985 Sensory effects of transient electrical stimulation—evaluation with a neuroelectric model IEEE Trans. Biomed. Eng. 32 1001–11

[9] Carlon P F, van Wieringen A, Deeks J M, Long C J, Lyzenga J and Wouters J 2005 Effect of inter-phase gap on the sensitivity of cochlear implant users to electrical stimulation Hear. Res. 205 210–24

[10] Cappaert N L M, Ramekers D, Martens H C F and Wadam W J 2012 Efficacy of a new charge-balanced biphasic electrical stimulus in the isolated sciatic nerve and the hippocampal slice Int. J. Neural Syst. 23 1250031

[11] Foutz T J and McIntyre C C 2010 Evaluation of novel stimulus waveforms for deep brain stimulation J. Neural Eng. 7 056008

[12] Haji Ghaffari D et al 2020 The effect of waveform asymmetry on perception with epiretinal prostheses J. Neural Eng. 17 045009

[13] Deprez M, Luyck Y, Luyten L, Tambyuser T, Nuttin B and Mc Laughlin M 2018 An evaluation of the effect of pulse-shape on grey and white matter stimulation in the rat brain Sci. Rep. 8 752

[14] Randj B J 1975 Which elements are excited in electrical stimulation of mammalian central nervous system: a review Brain Res. 98 417–40

[15] Basser P J and Roth B J 2000 New currents in electrical stimulation of excitable tissues Annu. Rev. Biomed. Eng. 2 377–97

[16] Rattay F 1989 Analysis of models for extracellular fiber stimulation IEEE Trans. Biomed. Eng. 36 676–82

[17] Durand D M 2000 Electrical stimulation of excitable tissue Biomedical Engineering Fundamentals 2nd edn, ed J D Bronzino (Boca Raton: CRC Press) pp 248–69

[18] Volkman J, Moro E and Palwa R 2006 Basic algorithms for the programming of deep brain stimulation in Parkinson’s disease Mov. Disord. 21 S284–9

[19] Lozano A M et al 2019 Deep brain stimulation: current challenges and future directions Nat. Rev. Neurol. 15 140–60

[20] Kringlebch M L, Jenkinson N, Owen S L and Aziz T Z 2007 Translational principles of deep brain stimulation Nat. Rev. Neurosci. 8 623–35

[21] Gradinaru V, Mogri M, Thompson K R, Henderson J M and Deisseroth K 2009 Optical deconstruction of parkinsonian neural circuitry Science 324 354–9

[22] Nowak L G and Bullier J 1998 Axons, but not cell bodies, are activated by electrical stimulation in cortical gray matter: Evidence from selective inactivation of cell bodies and axon initial segments Exp. Brain Res. 118 489–500

[23] McIntyre C C and Grill W M 1999 Excitation of central nervous system neurons by nonuniform electric fields Biophys. J. 76 878–88

[24] Buzsáki G 2006 Rhythms of the Brain (Oxford: Oxford University Press)

[25] Jensen A L and Durand D M 2009 High frequency stimulation can block axonal conduction Exp. Neurol. 220 57–70

[26] Feng Z, Zheng X, Yu Y and Durand D M 2013 Functional disconnection of axonal fibers generated by high frequency stimulation in the hippocampal CA1 region in-vivo Brain Res. 1509 32–42

[27] Guo Z, Feng Z, Wang Y and Wei X 2018 Simulation study of intermittent axonal block and desynchronization effect induced by high-frequency stimulation of electrical pulses Front. Neurosci. 12 858

[28] Wang Z, Feng Z, Yuan Y and Zheng L 2021 Suppressing synchronous firing of epileptiform activity by high-frequency stimulation of afferent fibers in rat hippocampus CNS Neurosci. Ther. 27 352–62

[29] Yu Y et al 2016 Modulation of local field potentials by high-frequency stimulation of afferent axons in the hippocampal CA1 region J. Integr. Neurosci. 15 1–17

[30] Richardson T L, Turner R W and Miller J J 1987 Action-potential discharge in hippocampal CA1 pyramidal neurons: current source-density analysis J. Neurophysiol. 58 981–90

[31] Feng Z, Yu Y, Guo Z, Cao J and Durand D M 2014 High frequency stimulation extends the refractory period and generates axonal block in the rat hippocampus Brain Stimulation 7 680–9

[32] Rattay F 1999 The basic mechanism for the electrical stimulation of the nervous system Neuroscience 89 335–46

[33] Deutsch J A 1964 Behavioral measurement of the neural refractory period and its application to intracranial self-stimulation J. Community Psychol. 58 1–9

[34] Farmer T W, Buchthal F and Rosenfalk P 1960 Refractory period of human muscle after the passage of a propagated action potential Electroencephalogr. Clin. Neurophysiol. 12 455–66

[35] Iremonger K J, Anderson T R, Hu B and Kiss Z H 2006 Cellular mechanisms preventing sustained activation of cortex during subcortical high-frequency stimulation J. Neurophysiol. 96 613–21

[36] Zheng F, Lammert K, Nixdorf-Bergweiler B E, Steigerwald F, Volkmann J and Alzheimer C 2011 Axonal failure during high frequency stimulation of rat subthalamic nucleus J. Physiol. 589 2781–93

[37] Shin D S, Sanoilova M, Cotic M, Zhang L, Brotchie J M and Carlen P L 2007 High frequency stimulation or elevated K+ depresses neuronal activity in the rat entopueduncular nucleus Neuroscience 149 68–86

[38] Bellinger S C, Miyazawa G and Steinmetz P N 2008 Submyelin potassium accumulation may functionally block subsets of local axons during deep brain stimulation: a modeling study J. Neural. Eng. 5 263–74

[39] Warman E N, Grill W M and Durand D 1992 Modeling the effects of electric fields on nerve fibers: determination of excitation thresholds IEEE Trans. Biomed. Eng. 39 1244–54

[40] Mino H, Rubinstein J T, Miller C A and Abbas P J 2004 Effects of electrode-to-fiber distance on temporal neural response with electrical stimulation IEEE Trans. Biomed. Eng. 51 13–20

[41] Samoudi A M et al 2017 Numerical modeling of percutaneous auricular vagus nerve stimulation: a realistic 3D model to evaluate sensitivity of neural activation to electrode position Med. Biol. Eng. Comput. 55 1763–72

[42] Kloosterman F, Peloquin P and Leung L S 2001 Apical and basal orthodromic population spikes in hippocampal CA1 in vivo show different origins and patterns of propagation J. Neurophysiol. 86 2435–44

[43] Shepherd R K and Iavel E 1999 Electrical stimulation of the auditory nerve: II. Effect of stimulus waveform on single fibre response properties Hear. Res. 130 171–88