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High Frequency Stimulation Selectively Blocks Different Types of Fibers in Frog Sciatic Nerve

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Abstract

Conduction block using high frequency alternating current (HFAC) stimulation has been shown to reversibly block conduction through various nerves. However, unlike simulations and experiments on myelinated fibers, prior experimental work in our lab on the sea-slug, *Aplysia*, found a nonmonotonic relationship between frequency and blocking thresholds in the unmyelinated fibers. To resolve this discrepancy, we investigated the effect of HFAC waveforms on the compound action potential of the sciatic nerve of frogs. Maximal stimulation of the nerve produces a compound action potential consisting of the A-fiber and C-fiber components corresponding to the myelinated and unmyelinated fibers' response. In our study, HFAC waveforms were found to induce reversible block in the A-fibers and C-fibers for frequencies in the range of 5–50 kHz and for amplitudes from 0.1–1 mA. Although the A-fibers demonstrated the monotonically increasing threshold behavior observed in published literature, the C-fibers displayed a nonmonotonic relationship, analogous to that observed in the unmyelinated fibers of *Aplysia*. This differential blocking behavior observed in myelinated and unmyelinated fibers during application of HFAC waveforms has diverse implications for the fields of selective stimulation and pain management.

Index Terms

Compound action potential; A-fiber; C-fiber; selective stimulation; pain

I. INTRODUCTION

One of the major challenges in designing effective neural prosthetic systems is stimulating specific fibers analogous to the physiological recruitment order without affecting the functionally irrelevant fibers. Selective stimulation of specific fibers is essential to restore functionality and can be achieved by temporarily blocking the conduction of signals through the extraneous fibers. Arresting or blocking superfluous activity through peripheral nerves can also be useful for alleviating disease symptoms and eliminating the debilitating nature of various neuromuscular pathologies, like dystonia, dyskinesia, and spasticity. These pathological conditions commonly involve neuronal hyperactivity that cause undesirable sensations and hinder dexterous motor control. Selective blocking of afferent activity in specific fibers is also desirable for conditions associated with chronic pain, like in neuromas and neuralgias. Various thermal, mechanical, surgical and pharmacological methods have been used for selective blocking but are unsuitable for chronic clinical applications, since they are not quick acting and quick reversing, are non-specific and can possibly cause irreversible nerve damage[1].

The application of high frequency alternating current (HFAC) waveforms on peripheral nerves has been found to be a potential clinical method for blocking conduction of action potentials through nerves [2] and achieving selective stimulation [3]. HFAC waveforms in the range of 1–40 kHz have been shown to induce complete and reversible local block in whole nerves [1, 2, 4–7]. The block threshold, defined as the amplitude of the HFAC waveform below which complete block did not occur, was found to monotonically increase with frequency in myelinated animal model systems of frog, rat and cat nerves, where muscle force was used as an indirect measure of block status [1, 4–6]. Traditionally only the myelinated response of the nerve and its effect on muscle force has been studied. Simulation studies showed that smaller diameter axons have higher blocking thresholds than the larger diameter axons at the same frequency [4, 5, 8–10] but the effect of HFAC waveforms on the smaller diameter, slower conducting unmyelinated fibers has not been experimentally validated.

Experimental work in our lab on the sea-slug, *Aplysia californica*, showed that HFAC stimulation could induce complete and reversible conduction block in unmyelinated fibers for frequencies in the range of 5–50 kHz and that compound action potential recordings could be used as a reliable method for monitoring block status[7]. Although, the characteristics of the neural activity during HFAC stimulation in these unmyelinated nerves mimicked the characteristics of the myelinated nerves previously observed in literature, the minimum amplitude for inducing block in these nerves decreased for frequencies above 12 kHz, as shown in Figure 1. This nonmonotonic block threshold behavior was found to be a property of the nerve and not an artifact of the experimental setup. If this disparity in the block threshold behavior at higher frequencies exists between myelinated and unmyelinated fibers, then distinct zones for selective stimulation of specific fiber type populations can be found on the frequency-amplitude spectrum that would have several applications in the fields of neural prostheses and pain management.

The ability to block the smaller diameter unmyelinated pain fibers while allowing conduction through the larger diameter myelinated fibers would provide a novel means for selective blocking. To validate our results obtained from the *Aplysia* nerves and investigate whether the nonmonotonic blocking response of unmyelinated fibers is unique only to the *Aplysia* nerves or is more generally applicable, we studied the effect of HFAC stimulation on the compound action potential (CAP) of amphibian mixed nerves. The sciatic nerve of frogs, frequently used in experimental studies, is a mixed nerve composed of myelinated and unmyelinated fibers. Investigating the effect of HFAC waveforms on the different components of the CAP would enable us to detect the progression of block in each fiber type population within the whole nerve.

In this paper, we describe the *in vitro* experiments performed on the sciatic nerve of the frog where neural activity was directly monitored using CAP recordings, to determine the block status. For simplicity, we grouped the fast-conducting myelinated fibers with conduction velocities greater than 20m/s as the A-fiber response and the slow-conducting unmyelinated fibers with conduction velocities less than 1m/s as the C-fiber response. If the A-fiber and C-fiber components of the CAP are found to have different block threshold behaviors at different frequencies, then this method of block induction could be significant for a variety of clinical and neurophysiological applications.

II. MATERIALS AND METHODS

A. Animal Preparation

In vitro acute experiments were performed on the sciatic nerve of nine leopard frogs, *Rana pipiens*. Prior to surgery, the frogs were anesthetized with tricainemethanesulfonate

(MS-222, 1 g/L) and then the frogs were double pithed. The sciatic nerve was exposed along its entire length through a dorsal incision and cut at the level of the spinal cord. The nerve, usually about 5 cm long, was ligated at both ends with silk threads. The threads were pinned to a petri dish with a Sylgard base (Dow Corning), and the dish was filled with normal frog Ringer's solution (NaCl 83.89mM, NaHCO₃ 28.11 mM, KH₂PO₄ 1.2mM, KCl 1.5mM, MgSO₄ 1.2mM, CaCl₂ Dihydrate 1.3mM, Glucose 10 mM, pH adjusted to 7.4). The frogs were decapitated at the end of the dissection. The preparation usually lasted for about 4 hours on average. All experiments were conducted at room temperature and all protocols involving animal use were approved by the Georgia Tech Institutional Animal Care and Use Committee.

B. Electrophysiological setup

Glass suction electrodes, with tip diameters (inner diameter) about the same as that of the whole sciatic nerve (0.5–1 mm), were used in our experiments. Figure 2 illustrates the experimental setup used for recording CAPs triggered in the sciatic nerve during application of the HFAC waveforms. This experimental setup was similar to that previously used for the *Aplysia* nerves [7]. Two electrodes were used for recording the propagation of the CAP along the nerve. One electrode was used to trigger a CAP while another electrode, placed along the nerve, was used to provide the block-inducing HFAC waveform. The distance between the electrodes was optimized to maximize the separation between the stimulus artifact and the recorded CAP while enabling detection of the distinct peaks of the different components of the CAP. Pilot tests were conducted to determine the optimum distance between the electrodes that enabled sufficient separation of the stimulus artifact and the A-fiber and C-fiber components of the CAP while preventing temporal dispersion of the CAP. Large electrode separation distances would enable separation of the stimulus artifact and the different components of the CAP. However, larger distances increase the temporal dispersion of the CAP, which decreases the amplitude of the different components of the CAP. This in turn makes it extremely difficult to isolate the smaller amplitude C-fiber component of the CAP from the baseline noise, which is critical to identify block status. A distance of about 5 mm between the AP triggering electrode and the first recording electrode was found to be optimum for the nerves in this study. The second recording electrode was placed about 10 mm from the first recording electrode and the blocking electrode was placed midway between the recording electrodes. The propagation of impulses along the nerve was used as an output measure to monitor block status. A suprathreshold bipolar stimulus pulse of 10V for 0.2ms, converted to current stimulation (0.1mA/V), through a stimulus isolation unit (AM Systems-Analog Stimulus Isolator, Model 2200, Carlsborg, WA) was used to trigger the CAP. High frequency sinusoidal waveforms generated by a function generator (Stanford Research Systems, Model DS345) and sent through a similar stimulus isolation unit (0.1mA/V) were used for block induction.

The signal was differentially amplified with a gain of 1000 for the A-fiber response and a gain of 10K for the C-fiber response. The bandwidth was limited to 100 Hz – 5 kHz to minimize interference during application of the high frequency waveforms. In some cases, additional post-data filtering in Clampfit (bandpass filter = 100 Hz – 3 kHz), enabled identification of block status of the C-fiber components. These optimal settings filtered out the noise from the high frequency waveforms and enabled easy recognition of block status of the different components of the CAP. The sampling rate of the data acquisition system was 50 kHz. Our experimental set up had the advantage of providing direct monitoring of the neural activity along the nerve, unlike other published studies where muscle force or sphincter pressure was used as an indirect measure of nerve block.

C. Experimental Procedures

The sciatic nerve was stimulated and the A-fiber and C-fiber components were isolated from the triggered CAP. Repeated, randomized experiments were conducted on each nerve for different frequencies in the range of 5–50 kHz and amplitudes in the range of 0.1–1 mA to characterize the activity of the different components of the CAP during application of the HFAC waveforms. 50 kHz was the maximum frequency tested in our study due to slew rate limitations of the equipment. It must be noted that it may be possible to achieve block above 50 kHz, but for the purposes of our study, we limited the range to 50 kHz. In each trial, the HFAC stimulus was applied on the nerve and then a test pulse was injected to trigger a CAP. Each trial consisted of an average of 20 successive runs to cancel out the asynchronous noise for detection of the C-fiber component. Each run was about 1 s each in duration. The CAP trigger stimulus was provided 15 ms into each run. The nerve was allowed to rest for at least a minute between successive trials. If the amplitude of the HFAC waveform was at or above the threshold for inducing conduction block, the CAP was arrested at the site of injection of the blocking stimulus. The minimum amplitude of the HFAC waveform at which the CAP was not observed in the distal recording electrode was identified as the ‘block threshold’. A repeat trial of each identified block threshold was conducted randomly during the experiment to verify the threshold value for a particular frequency.

Block thresholds of the A-fiber and C-fiber component of the CAP for at least 2 different frequencies were determined for each nerve. To identify block thresholds, multiple trials were conducted for each randomly chosen frequency in the range of 5–50 kHz. On average at least 80 trials were obtained from each nerve. The response of the nerve before, during and after application of HFAC waveforms was recorded. A range of amplitudes was tested to identify the threshold at which block was observed. The amplitude was incremented in discrete steps initially of 0.1–0.3 mA and then of 0.01–0.05 mA closer to block threshold. These multiple trials at each frequency enabled completed characterization of the response of the nerve to different frequencies and amplitudes of the HFAC waveform in the chosen range.

The response during application of the HFAC waveform was compared with that prior to and after application of the high frequency stimulation. The presence or absence of the different components of the CAP was used to determine block status of each component. The nerve response was classified as ‘No change’, ‘Partial Block’ and ‘Block’, based on the amplitude of the component of recorded CAP during application of the high frequency waveforms. This classification was done both for the C-fiber and the A-fiber components of the CAP. ‘No change’ was identified as the amplitudes of HFAC stimulation when the CAP component was similar to the amplitude of CAP component prior to switching on the HFAC waveform. ‘Partial block’ was identified as the amplitudes below the block threshold where the component of the CAP was less than 50% of the amplitude of the component prior to switching on the HFAC waveform. ‘Block’ was identified as the amplitudes of the HFAC waveform when the component of the CAP was less than 10% of the CAP component prior to switching on the HFAC waveform.

III. RESULTS

All experimental preparations were initially tested for normal conduction properties to determine if a CAP could be repetitively triggered and transmitted along the axon. The triggered CAP appeared in the two recording electrodes with a latency equal to the propagation delay through the nerve. The conduction velocities were greater than 20 m/s for the A-fiber component and in the range of 0.4–1 m/s for the C-fiber components. The stimulus artifact also appeared in the recording traces but was usually well separated from the A-fiber component of the CAP in the second recording electrode due to the longer

distance from the stimulating electrode. The C-fiber component of the CAP, due to its slower conduction velocity, appeared at a latency greater than 20ms after the A-fiber response. Detection of these waveforms was essential for determining the A-fiber and C-fiber block thresholds. A typical CAP recording from which the A-fiber and the C-fiber components were extracted is shown in Figure 3.

Conduction block induced by HFAC stimulation was demonstrated in all nerves tested. In each nerve, block thresholds could be obtained for at least 2 different frequencies in the range of 5–50 kHz. In the absence of the HFAC and for stimulus intensities below the block thresholds, normal conduction of the CAP was observed. In cases where conduction block could be observed, CAP conduction returned within a few seconds of switching off the high frequency current and was instantaneous in some cases. Reversibility of conduction and recovery of the CAP components to their pre-block amplitudes were a requisite condition for the admissibility of the data. Failure of recovery of the CAP to its pre-block amplitude and shape after the HFAC stimulus was switched off, was attributed to improperly constructed electrodes, electrodes that had a degraded AgCl coating, a weak battery in the stimulus isolator or degrading health of the nerve preparation. If complete recovery was not seen, the experiment was terminated and the last dataset was eliminated. If the block thresholds for at least two different frequencies were not obtained then the entire data collected from the nerve was not admitted. We were able to obtain block thresholds for at least 2 different frequencies from 9 nerves with an average of 80 trials. In 2 nerves, the experiment had to be terminated early, after only 50 trials, because the C-fiber component of the CAP could not be differentiated from the baseline noise.

The neural activity of the nerve changed depending on the amplitude and frequency of the HFAC waveform. A decrease in the amplitude or a notable change in the shape of the CAP, during application of the HFAC waveform, was indicative of block of only few axons in the nerve. In some cases, 100 % block of the A-fiber component could not be obtained and is hypothesized to be due to the incomplete encircling of nerve by the electrode as has been previously reported [4, 11]. Figure 4 displays sample traces of the relative amplitudes of the C-fiber component of CAP when ‘partial block’ and ‘block’ were identified for different frequency and amplitude combinations during application of HFAC waveforms. Partial block always occurred at amplitudes of the HFAC waveform that were immediately below those amplitudes where complete block of neural activity was observed (Fig. 5).

Data obtained from all the trials was pooled and a mapping of the neural activity of the A and C-fiber components at different frequencies and amplitudes is plotted in Figure 6. The current intensity required to block conduction increased with frequency for the myelinated nerves (Figure 6A), while for the unmyelinated nerves, it increased until about 20 kHz and then decreased until 50 kHz, which was the maximum frequency tested (Figure 6B). The minimum amplitude of the HFAC waveform for inducing block at a particular frequency was termed the ‘block threshold’. The average block thresholds for the A and C-fiber components obtained from all the nerves tested at different frequencies in the range of 5–50 kHz is shown in Figure 7. The error bars indicate the standard error of means (SEM) at each frequency. The sample size was greater than 5 for all frequencies tested except for the frequencies where the block thresholds were close to 1 mA (15 kHz, and 30 kHz for C-fibers, and 30 kHz for A-fibers). We specifically chose SEM because the sample sizes were not identical for all the frequencies tested, and SEM provides a visual measure of how reliable our estimate of the mean is. The minimum amplitudes of the HFAC waveform required to induce conduction block in the A-fiber component of the CAP increased with frequency, while in the C-fiber component of the CAP a nonmonotonic behavior with frequency was observed. The average block thresholds for the A-fiber component of the CAP could be fit to a logarithmic curve ($R^2=0.99$), while the average block thresholds for

the C-fiber component of the CAP could be approximated to a third order polynomial ($R^2=0.96$). The nonmonotonic block threshold behavior of the C-fiber component and the linear block threshold behavior of the A-fiber component of the CAP create two distinct regions on the frequency-amplitude spectrum where a specific component of the CAP can be selectively blocked. In certain cases where the block thresholds of the A and C-fiber components were close to each other, a reduction in the amplitude of the non-blocked component was observed for amplitudes above the block threshold of the blocked component. Figure 8 displays sample traces where selective block of either component of the CAP could be obtained by choosing a specific amplitude and frequency.

IV. DISCUSSION

This paper is the first study to experimentally explore the frequency-amplitude relationship of the different components of the CAP of a mixed nerve to HFAC stimulation. The results show that HFAC waveforms can induce conduction block in whole nerves for frequencies from 5–50 kHz and amplitudes from 0.1–1 mA. This is the first study to investigate the effect of HFAC waveforms on the unmyelinated C-fibers and demonstrate that the behavior of these unmyelinated fibers differs from that of the fast-conducting myelinated fibers. It is also the first to demonstrate that the unmyelinated and myelinated components of the mixed nerve can be consistently, repeatedly and separately blocked in peripheral amphibian nerves.

Our experimental set-up, using direct measures of nerve activity through compound action potential recordings, offers a powerful technique to investigate the effect of HFAC waveforms on the different fiber types in mixed nerves and identify regions where specific fiber types can be selectively blocked. Direct measurements of neural activity through CAP recordings and single fiber recordings have previously been used to study the effect of HFAC waveforms on nerves. [2, 7, 12–14]. Most of the recent studies have been focused on observing motor outputs using muscle twitch force and external urethral pressure to study the phenomena [1, 4–6, 15, 16]. However, studies using motor outputs cannot capture the effect of HFAC waveforms on different fiber types or fiber diameters. Behavior observed through CAP recordings obtained during application of HFAC waveforms was consistent with the behavior observed through motor outputs. In addition, observing partial and complete block of the different components of the CAP provides information about the progression of block in different fiber types in whole nerves. Specifically, CAP recordings can be advantageous in isolating the effect of HFAC waveforms on sensory nerves, which can be a valuable tool for applications related to pain management.

This technique would also enable the interpretation of the relative contributions of distinct fiber types to different functions in the nervous system and to differentiate the response of smaller and larger diameter fibers in neural pathways.

Published work on HFAC block has shown that in motor nerves, the block threshold increases with frequency. Computational studies have showed that the block threshold using HFAC waveforms is inversely proportional to axon diameter [4, 5, 8–10], and have postulated that the smaller diameter unmyelinated fibers should have a higher block threshold than the larger diameter myelinated fibers at all frequencies [8, 9]. However, in our experiments a pure diameter dependence of threshold on frequency was not observed. While the block thresholds of the larger diameter myelinated fibers continually increased with frequency, the block thresholds of the smaller diameter unmyelinated fibers decreased as frequency increased above 30 kHz. The A-fiber component of the CAP showed a strong linear relationship with frequency consistent with previously published experiments on myelinated nerves [1, 4–6] but the C-fiber component of the CAP displayed a nonmonotonic relationship that contradicted hypothesized results from computational studies [8, 9] but

validated the results previously obtained on the purely unmyelinated nerves of the sea-slug *Aplysia* [7]. Since the frequencies were tested in a random order and since the nonmonotonic relationship was observed only in the C-fiber component of the CAP and not in the A-fiber component (Figure 7 and Figure 8B), it can be concluded that the negative slope relationship observed at higher frequencies is an inherent property of unmyelinated nerves and not an artifact of the experimental set-up or nerve fatigue.

The average block thresholds for different fiber types are markedly distinct at certain frequencies, as evident in Figure 7. Our study demonstrates that selective block of either the A-fiber component of the CAP (Fig. 8A) or the C-fiber component of the CAP (Fig. 8B) can be obtained by choosing the appropriate frequency and amplitude combination. In our experiments, block thresholds for some frequencies could not be precisely determined due to physical limitations of the equipment that restricted the maximum current output to 1 mA. We acknowledge that this limits the accuracy of the extrapolated block thresholds at those frequencies, but other features of nerve activity, like observing partial block (Figure 5) or no change in the features of the CAP for amplitudes below 1 mA, indicate that the block thresholds at those frequencies were above 1mA. Nerve block might also be obtainable over a wider frequency range for amplitudes that could not be tested with our present apparatus and future work is aimed at expanding this range.

Block induction using HFAC waveforms is associated with an onset response and a period of asynchronous firing, just prior to block induction. This onset response is known to increase initially and then decrease as the amplitude or frequency of the waveform increases [4, 7, 17, 18]. Elimination or reduction of the onset response observed prior to block induction will be critical if clinical applications utilizing HFAC waveforms for conduction block are to be pursued and various investigations are currently underway to develop methods to circumvent this response [19–22]. In our current study, we did not observe the repetitive firing activity since the recorded data was the average of 20 runs. During clinical implementation of the HFAC block induction technique, it is possible that depending on the chosen amplitude and frequency, one fiber type may be in the asynchronous firing phase or partial block phase while the other is in the complete block phase. Hence, in order to obtain selective blocking without affecting the extraneous fibers, frequencies with maximum separation of the block thresholds, should be chosen. Additionally, higher frequencies (>30 kHz) were found to have minimum onset properties [4, 7, 19] and hence would be the preferred frequency regime for clinical applications related to selectively blocking C-fibers.

Prior simulation work has indicated that the frequency of the waveform, the computational model used, and the possible interactions between the nodes of Ranvier, are key issues in achieving the localized electrical nerve block and these factors could affect the block threshold at each frequency [5, 9, 10, 23, 24]. Yet in all these computational studies a linear relationship with frequency was found even for smaller diameter axons. A possible explanation for the discrepancy with our experimental results is that these studies have been based on axon models that are not relevant for the nerves tested. Simulation work in our lab has attempted to understand the decrease in block thresholds at higher frequencies and a modified Hodgkin-Huxley (HH) model with a frequency-dependent capacitance, based on data from squid axons, showed a deviation from the linear threshold behavior [25, 26]. The HH model assumes that capacitance is constant even at higher frequencies when actually, experimental data from the giant squid axon shows that capacitance decreases as frequencies increase [27]. Incorporating this frequency-dependent capacitance into a HH model showed a non-linear block threshold response, but the nonmonotonic block threshold response observed experimentally could not be replicated. However, we note that the HH model was developed for large diameter unmyelinated axons (500–1000 μm in diameter) while the diameter of axons in our experiments were orders of magnitude smaller (< 5 μm in

diameter). Additionally, the HH model simulates the behavior of a single axon, while data from our experiments was obtained from nerves containing thousands of axons.

Computer simulations demonstrating block induction via HFAC waveforms have shown that during application of the HFAC waveforms the membrane voltage increases dramatically beyond the physiological range. The models studying the phenomena however have not been validated for those membrane voltage regimes. Another consideration is that most of the computational studies investigating the mechanism of conduction block induced by HFAC waveforms have only looked into the ion channel gating mechanisms that prevent action potential propagation during application of the HFAC waveforms. The surrounding extracellular environment that plays a significant role in enabling axonal conduction has been ignored in these models. The drastic changes in the membrane voltages during application of the HFAC waveforms could lead to imbalances in the $\text{Na}^+\text{-K}^+$ pumps, which could lead to an accumulation of K^+ in the extracellular environment and impede action potential propagation through the nerve. Current computational models studying HFAC block induction do not account for these extracellular changes. Our previous study [7], found that conduction block is also dependent on the duration of application of the HFAC waveform, suggesting that even though initially an ionic gating mechanism may be responsible for block induction, secondary mechanisms prevent instantaneous recovery after removal of the HFAC waveform. We hypothesize that the differences in the active and dielectric membrane properties, binding kinetics and accumulation of ions in the axoplasm as well as the surrounding myelin sheath may contribute to the disparity in the behavior of different fiber types of mixed nerves. It is quite possible that depending on the frequency and the amplitude of the HFAC waveform and fiber type, different or multiple mechanisms might be responsible for block induction. Significant experimental and simulation work will be required to gain a complete understanding of these differences.

This study demonstrates that HFAC waveforms can potentially be used to exploit inherent nerve properties for selectively blocking conduction through specific types of fibers while enabling conduction through the complementary fibers. For this study, we only investigated the differences in the blocking thresholds of the fast-conducting myelinated fibers and the unmyelinated fibers and a similar setup can be used to evaluate the effect of HFAC waveforms on other fiber type populations. Future studies are aimed at understanding this phenomenon in greater detail, such as investigating how the A-delta fibers and the B fibers respond to high frequency stimulation, and whether the observed non monotonic behavior is a property of the fiber type or of fiber diameter. However, discriminating between the different components of the CAP during application of the HFAC waveforms can be challenging due to the close proximity of the frequency of the HFAC waveforms and the bandwidth of interest of the various components of the CAP. Selective blocking of conduction through the pain fibers has been the goal of many researchers and our study shows that HFAC waveforms may possibly be used to selectively and reversibly induce block in the C-fibers, while enabling conduction through the A-fibers. Future work is also aimed at demonstrating that frequency modulation of the HFAC waveform may enable the selective blocking and unblocking of fibers of varying diameters to provide more control on the type of stimulation given to restore functionality, thus improving the current state of extracellular stimulation in neural prostheses.

V. CONCLUSION

This study characterizing the effect of HFAC waveforms on individual fiber type populations of mixed nerves has demonstrated that larger diameter myelinated fibers and smaller diameter unmyelinated nerves fibers have different responses to HFAC stimulation and the threshold behavior is non-uniform across the frequency range of 5–50 kHz. While

we were unable to accurately identify the block thresholds at certain frequencies, other features of the components of the CAP helped deduce the behaviors of block thresholds for the entire range. This is the first study to describe the effect of HFAC waveforms on different fiber types in mixed nerves using compound action potential recordings. It is also the first study to demonstrate that unmyelinated nerves can be selectively blocked using HFAC waveforms, while maintaining conduction in the myelinated fibers. The nonmonotonic threshold behavior of the unmyelinated fibers compared to the linear threshold behavior of myelinated fibers offers distinct frequency-amplitude combinations where specific fiber types can be selectively blocked or stimulated. These results are significant for potential clinical applications related to movement disorders and pain management. Future studies in our lab are aimed at identifying the response of other fiber types in mixed nerves to gain an understanding of the mechanisms causing the non-monotonic behavior displayed by the unmyelinated C-fibers and at addressing the limitations of the current study.

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Figure 1.

Non monotonic block threshold behavior observed in the purely unmyelinated nerves of the sea-slug, *Aplysia californica*. The minimum amplitude above which action potential conduction was blocked increased until about 12 kHz and then decreased until 50 kHz which was the maximum frequency tested. This non monotonic behavior deviated from the linearly increasing behavior previously observed in amphibian and mammalian nerves where the myelinated nerve response was studied. (Figure reproduced from [7]).

Figure 2. Schematic of experimental setup for recording and blocking the compound action potential from a frog sciatic nerve.

Compound Action Potential

Figure 3.

Typical recording of a compound action potential from which the A and C-fiber components can be extracted and analyzed. This recording is the average of 20 trials. The top trace indicates the recording over 80 ms. The green trace indicates a 10 ms time interval to view the stimulus artifact and the A-fiber response. The brown trace indicates a 40 ms time interval for viewing the C-fiber component of the CAP.

Figure 4.

Typical traces showing the C-fiber response, before, during and after application of HFAC waveforms. A: C-fiber component of the CAP prior to application of the HFAC waveform. B: Partial block was noted in certain cases when the amplitude of the component was less than 50% of the initial amplitude of the component. C: Changing the amplitude or the frequency of the HFAC waveform eliminated the component completely and this was noted as complete block of that component of the CAP. D: Switching off the HFAC waveform returned the C-fiber component of the CAP to an amplitude comparable to the initial amplitude of the component.

Figure 5.

Partial block always occurs at amplitudes below when block was observed. Trials on two different nerves showing amplitudes when partial block and block occurred in the C-fiber component of the CAP. The red squares indicate amplitudes at which conduction was blocked and the blue circles indicate amplitudes at which partial block was observed.

Figure 6.

Pooled data of all trials demonstrating when block occurred for the A-fiber and C-fiber components of the CAP for different frequencies and amplitudes of HFAC waveforms. A: The A-fiber response for different frequencies and amplitudes was plotted and a monotonically increasing trend can be observed. B: The C-fiber response for different frequencies and amplitudes was plotted and a nonmonotonic trend can be observed. The red squares indicate amplitudes at which the component was blocked. The black triangles indicate amplitudes at which the amplitude and shape of the CAP was similar to the CAP prior to application of the HFAC stimulus. Partial block amplitudes are not shown in this

figure but always existed at amplitudes between 'Block' and 'No Change' in individual trials, similar to the behavior observed in figure 5.

Figure 7.

Clustered column plots comparing the average block thresholds for A-fiber and C-fiber components of the CAP. The errors bars indicate the standard error of the means. The trend lines of the average block thresholds for each fiber type is also displayed. The block thresholds for the A-fibers proportionately increased with frequency while the block thresholds for the C-fibers increased and then decreased above 35 kHz. Average block thresholds at certain frequencies could not be accurately determined since they were above 1 mA, the maximum amplitude tested. The block thresholds of the A-fibers at different frequencies was fit to a logarithmic curve [$y = 0.4936\ln(x) + 0.0993$; $R^2 = 0.9914$] while the block thresholds of the C-fibers at different frequencies was approximated to a third order polynomial [$y = 0.0022x^3 - 0.0552x^2 + 0.3828x + 0.1944$; $R^2 = 0.9638$]. We note that there are two regions with different frequency-amplitude combinations, where one fiber type can be selectively blocked. For frequencies from 5–15 kHz, and amplitudes from 0.5–0.9 mA, only the A-fibers can be blocked without blocking the C-fibers and for frequencies from 35–50 kHz and amplitudes from 0.7–1 mA, only the C-fibers can be blocked without affecting the A-fibers.

Figure 8.

Selective block of the A fibers and C-fibers. Two different trials showing selective block of the components during application of HFAC waveforms. At low frequencies the A-fibers could be selectively blocked while at high frequencies, the C-fibers could be selectively blocked. A: Components of the CAP before application of HFAC waveform and during application of a 5 kHz waveform. The A-fiber component was blocked while the C-fiber component was not blocked during application of this 5 kHz HFAC waveform B: Components of the CAP before application of HFAC waveform and during application of a 35 kHz waveform. The A-fiber component was not blocked while the C-fiber component was blocked during application of this 35 kHz HFAC waveform demonstrating that HFAC

stimulation can be used to selectively block specific fiber types. In some other cases a reduction in amplitude of the non-blocked component was observed.