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Characterization of the high frequency alternating current block in the rat sciatic nerve using cuff electrodes and macro-sieve electrodes

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ABSTRACT OF THE THESIS

Characterization of the high frequency block in the rat sciatic nerve using cuff electrodes and macro-sieve electrodes for unidirectional signal transduction

by

Soumyajit Ray

Master of Science in Biomedical Engineering

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Research Advisor: Wilson Ray, Matthew MacEwan

Tripolar cuff electrodes were designed, fabricated and non-chronically implanted in the sciatic nerve of two-month-old Lewis rats. A proximal constant current stimulus to the nerve was blocked by applying a high frequency sinusoidal signal to the distally placed tripolar cuff electrode. The frequency voltage characteristic of the blocking signal was obtained. Complete block was not achieved using variants of the tripolar cuff design and bipolar cuff electrodes. Single and dual macro-sieve electrode assemblies were designed, fabricated and chronically implanted in the sciatic nerve of Lewis rats. After a four-month period for regeneration four different electrode configurations were tested to enable a high frequency block. A complete and quickly reversible block was obtained using both the macro-sieve electrodes for the HFAC block – proximal macro-sieve as anode and distal macro-sieve as cathode. Finite element modelling and axon modelling was done to determine the optimal parameters for effecting a high frequency block in the nerve.

Chapter 1

Introduction

Peripheral nerve interfaces devices enable us to stimulate nerves through external signals and therefore activate them. In order to gain complete control over the activation we also need a mechanism of inactivating or partially blocking / diminishing the signal. Specifically, there is a need to block the ante-retrograde transmission of the action potential in the sensory neurons when stim signals are specifically designed to activate distal musculature. Also, many diseases such as muscle spasms, spasticity, tics and chorea are characterized by unwanted hyperactivity of certain neurons which lead to pathological sensory or motor conditions. An effective mechanism of blocking the action potentials in these neurons can be used to alleviate the effects of the hyperactivity of these neurons.

1.1 Cuff electrodes

Neural interfaces can be classified as extraneural or intraneural. Extraneural devices include cuff electrodes (Veraart et al., 1993; Loeb and Peck,1996), slowly penetrating interfascicular nerve electrodes (SPINEs; Tyler and Durand, 1997), and flat-interface nerve electrodes (FINEs; Tyler and Durand, 2002). In this study, the cuff electrode with its wide-ranging applications, was used to demonstrate the high frequency block. As detailed in Chapter 2, a few different variants of the cuff electrode were designed and fabricated and the frequency voltage characterized to determine the relative efficacy in blocking a stimulus.

1.2 Macro-sieve electrodes

The macro-sieve electrode is a type of intraneural electrode (MacEwan et. al., 2016). Other intraneural devices include longitudinally implanted intrafascicular electrodes (LIFEs; Lawrence et al., 2003), the Utah electrode array (UEA) and the Utah slanted electrode array (USEA; Normann, 2007). Intraneural electrodes are characterized by their intimate contact with the interfaced nerves. Consequently, these electrodes have high recruitment specificity and low excitation thresholds (Yoshida and Horch, 1993; Branner et al., 2001; McDonnall et al., 2004a, b). The macro-sieve electrode has relatively large transit zones (as compared to other regenerative sieve electrodes) and a high transparency factor (MacEwan et. al., 2016) consequently limiting the impediment to motor fiber regeneration through the macro-sieve.

1.3 High Frequency Alternating Current (HFAC) block

A number of studies have demonstrated the use of a high frequency alternating current (HFAC) to affect a quick acting and rapidly reversible block (Bhadra et al. 2007, Tanner 1962; Woo and Campbell 1964; Bowman and McNeal 1986; Kilgore and Bhadra 2004; Tai et al. 2005a; Bhadra and Kilgore 2005; Williamson and Andrews 2005; Bhadra et al. 2006). The effect of the HFAC on the rat sciatic nerve had been detailed in Bhadra and Kilgore (2005). A range of frequencies (10kHz to 30kHz) were tested with a varying amplitudes of a sinusoidal block signal. For each frequency tested, the threshold amplitude, is defined as the lowest stimulus amplitude (p-p voltage) at which a complete block was observed. The threshold has been observed to increase linearly with the HFAC signal frequency (Figure 1.1). The HFAC is characterized by three phases; (a) and onset response (b) an asynchronous firing phase and (c) a blocking phase as shown in Figure 1.2

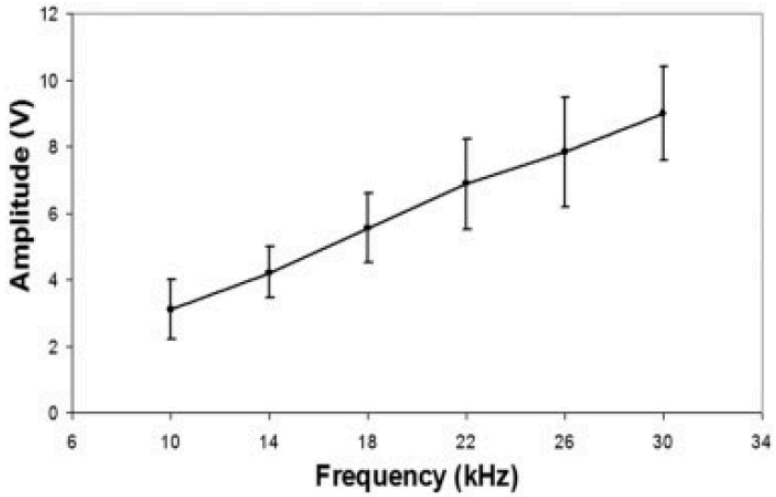


Figure 1.1: Relationship of block thresholds to frequency. (Each black dot is the average of 18 block threshold trials - three trials in each of 6 animals. Data shown with 1 SD.)
Source: Bhadra and Kilgore. "High-frequency electrical conduction block of mammalian peripheral motor nerve." *Muscle & nerve* 32, no. 6 (2005): 782-790.

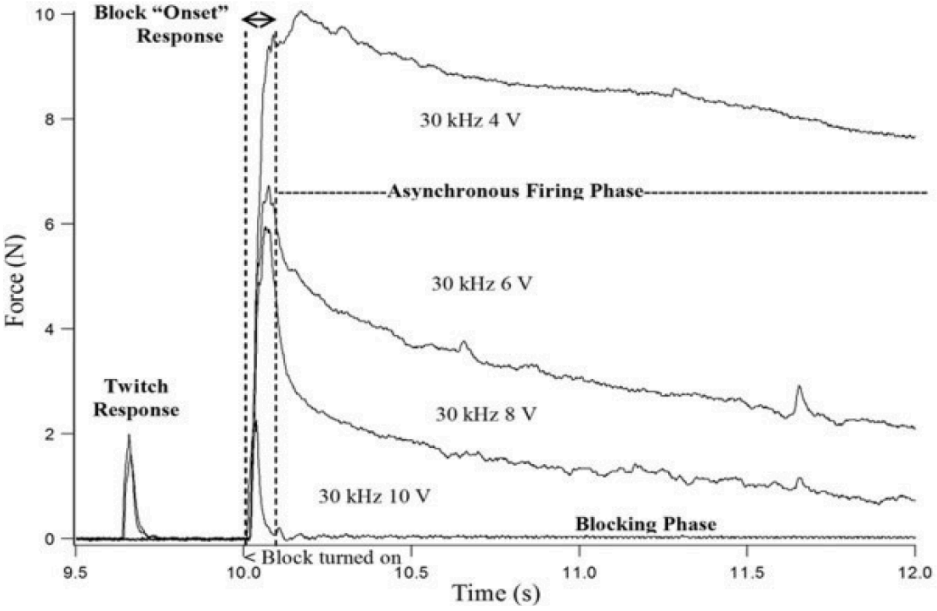


Figure 1.2: HFAC characteristics. Four trials from the randomized set in 1 animal using an HFAC at 30 kHz and four amplitudes. The block was turned on at 10 seconds in every trial. The three phases of high-frequency block are demonstrated. The block onset is shown followed by asynchronous firing at the lower amplitudes. At 10 V there was a block onset similar to a single muscle twitch, followed by complete block.
Source: Bhadra and Kilgore. "High-frequency electrical conduction block of mammalian peripheral motor nerve." *Muscle & nerve* 32, no. 6 (2005): 782-790.

Chapter 2

High Frequency Block Using Cuff Electrodes

2.1 Electrode design

2.1.1 Tripolar cuff electrode 1

This cuff electrode consisted of a central cathode and two anodes on either side (Figure 2.1). A silicone tube (Biomedical Silicone Tubing, AM Systems, #808500 Diameter: Inside 0.078 in., Outside 0.125 in.) of length 9mm was used to fabricate the electrode. A longitudinal cut was made on the tube to enable insertion of each electrode terminal. Each terminal consisted of a single length of multistrand silver wire (Medwire AG7/40T, 7 strands, diameter 0.25mm) across the internal circumference of the silicone tube. The silver wire was inserted 0.5mm from the longitudinal cut and exited on the other edge of the longitudinal cut, 0.5 mm from the cut, after traversing the internal circumference of the tube. The silver wire was held in place along the internal circumference of the tube using a single suture (Ethilon 6.0) diametrically opposite the longitudinal cut in the silicon tube. The outer surface of the tube was insulated using 1mm of medical grade elastomer (MDX4–4210, Dow Corning, Midland, MI). All three electrode terminals were constructed identically with a separation of 2mm between two adjacent terminals. The silver wires of the terminals were insulated using silicon tubing (SF medical, Part No. SFB21235, ID 0.032, OD 0.009) and the joints were insulated using the medical grade adhesive. The outer two terminal wires were connected together.

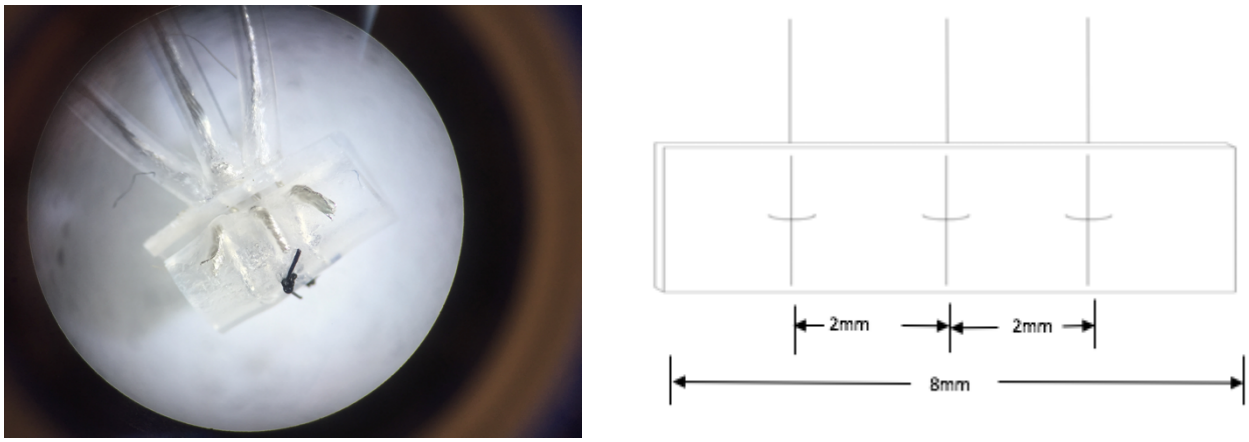


Figure 2.1: Ring type tripolar cuff electrode. Left panel – Image of the tripolar cuff electrode showing the three rings of each electrode terminal which go around the inner circumference of the silicone tube and the centrally placed suture to keep the terminals in place. Right panel – schematic of the tripolar ring type electrode. The distance between two adjacent electrode terminals is about 1mm.

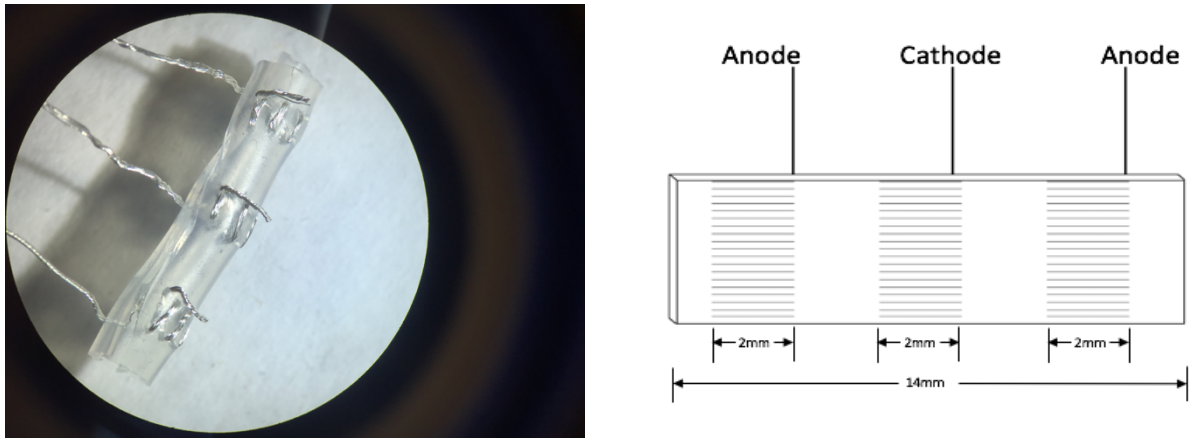


Figure 2.2: Ribbon type tripolar cuff electrode: Left panel – Image of the ribbon type tripolar cuff electrode showing the longitudinal 'stitches' in each terminal to increase the surface of contact with the nerve. Right panel – schematic of the flattened-out electrode showing the longitudinal stiches. The width of each electrode terminal is 2mm with a 2mm gap between two adjacent electrode terminals.

2.1.2 Tripolar cuff electrode 2

Similar to tripolar cuff electrode 1, this electrode consisted of a central cathode and two flanking cathodes. A biomedical silicone tubing (AM Systems, #808500 Diameter: Inside 0.078 in., Outside 0.125 in.) of length 14mm was used to fabricate the electrode. In order to increase the surface area of contact of the electrode terminal with the nerve, each electrode terminal consisted of the silver wire which was stitched into silicon tube across a 2mm length of the tube using longitudinal stitches (Figure 2.2). The rest of the construction was similar to tripolar cuff electrode 1 with the outer surface of the tube and the silver wires of each terminal insulated.

2.1.3 Tripolar cuff electrode 3

This electrode was identical in construction to tripolar cuff electrode 2 except that after the silver wire was stitched onto the silicone tube, silver epoxy was applied on the interior surface of the terminal to completely cover the silver wire. This was done to increase the surface area of contact with the nerve.

2.1.4 Bipolar cuff electrode

This electrode consisted of two terminals. Similar to electrode A, a silicone tube (Biomedical Silicone Tubing, AM Systems, #808500 Diameter: Inside 0.078 in., Outside 0.125 in., Wall Thickness 0.0235 in.) of length 4mm was cut longitudinally and each terminal was constructed using silver wire (Medwire AG7/40T, 7 strands, diameter 0.25mm) as described for tripolar cuff electrode 1. The outer surface of the tube was insulated using the medical grade adhesive and the terminal wires insulated using a separate biomedical silicone tube.

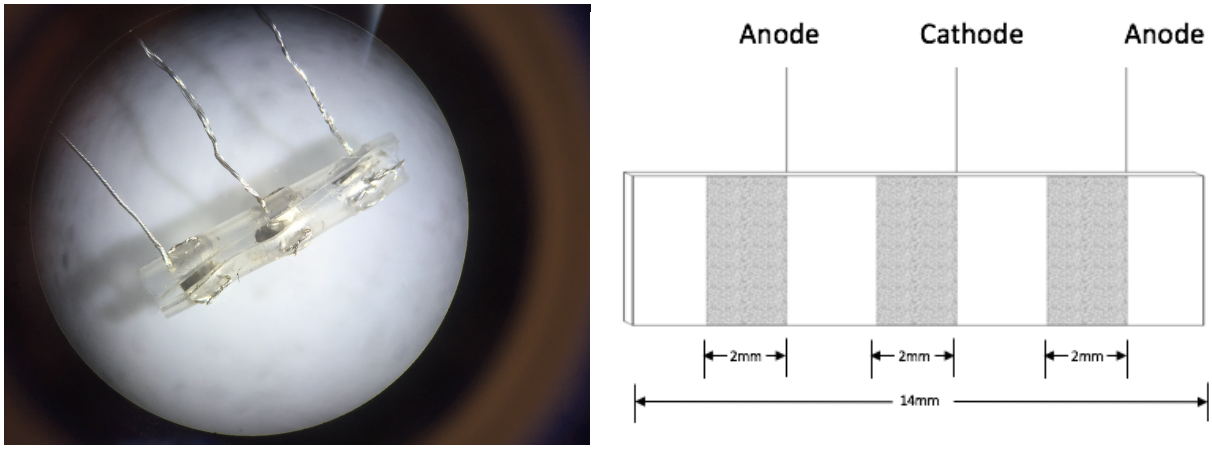


Figure 2.3: Ribbon type tripolar cuff electrode with silver epoxy: Left panel – image of the ribbon type tripolar cuff electrode with the silver epoxy. Right panel – schematic of the electrode showing the bands formed by the silver epoxy applied on top of the silver wires woven into the silicone tube.

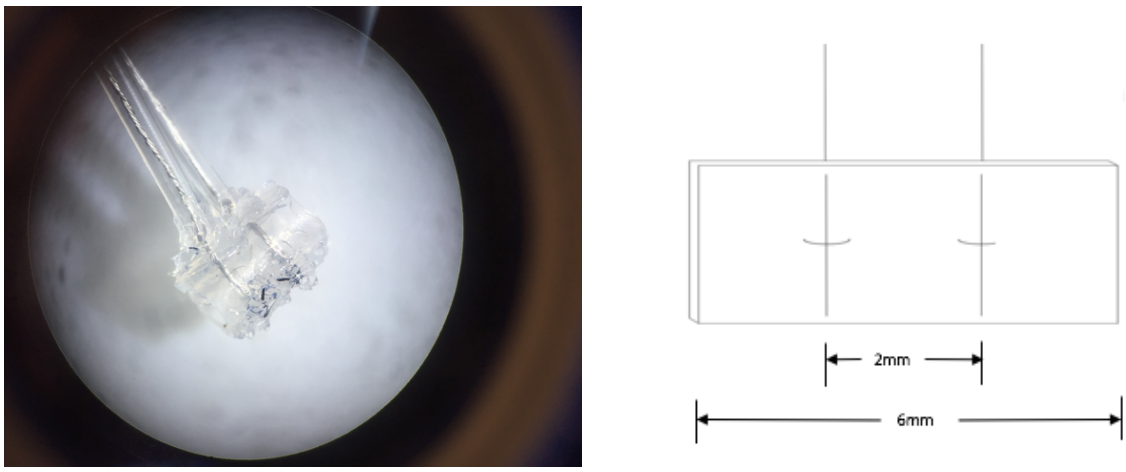


Figure 2.4: Bipolar cuff electrodes. Left panel – image of the bipolar cuff electrode with two rings of the wire as the electrode terminals. Right panel – schematic of the bipolar cuff electrode.

2.2 Experimental design

3 adult Lewis rats (weight range 340 – 360gms) were used for determining the frequency voltage characteristic of the HFAC block using cuff electrodes. All three types of tripolar cuff electrodes were used on each rat were used to determine the relative efficacy of each type of electrode.

2.3 Surgical procedure and setup

All surgical procedures were conducted with aseptic techniques. The Lewis rats were anesthetized by inhalation using 5% Isoflurane for induction and 2% for maintenance. For maintenance, the 2% Isoflurane was progressively reduced to 1.5% during the course of the surgery in order to reduce the detrimental effects of prolonged inhalation of Isoflurane. Following preparation of the incision site, the sciatic nerve was exposed to enable the placement of the cuff electrodes. A bipolar cuff electrode was placed proximally for generating electrically stimulated muscle response. The blocking electrode – a tripolar cuff electrode - was placed distal to this stimulation electrode and was used to generate the high frequency conduction block in the nerve (Figure 2.5). In some experiments, a third bipolar cuff electrode was placed distal to the tripolar cuff electrode. This electrode was used to demonstrate that the high frequency block does not result in fatigue at the neuromuscular junction since the muscle response can be generated through the stimulation of the distal electrode. The distal musculature was exposed to enable muscle force measurements. All the cuff electrodes were tied with a 6-0 Ethilon suture around the outer circumference of the silicon tube. This enabled a high contact surface area of the electrode terminal with the nerve. This was especially important for the blocking cuff electrode. Silicone oil was used to insulate the nerve and the cuff electrodes from the surrounding tissue. The surgical procedure of placing the electrodes on the sciatic nerve and evaluation of the high frequency block was done in a single session (electrodes were not chronically implanted).

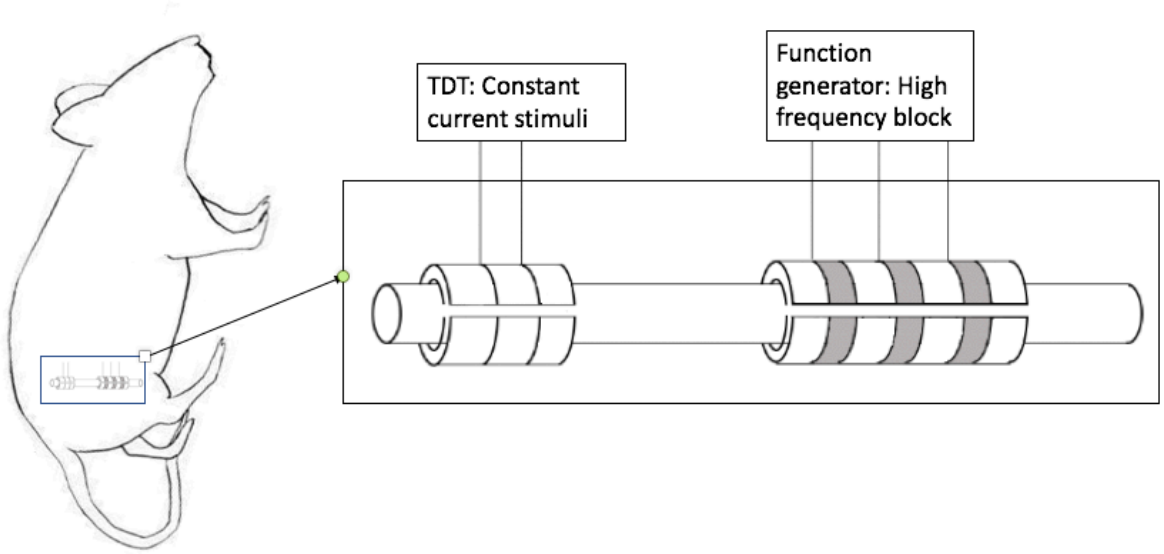


Figure 2.5: Experimental setup for cuff electrode based HFAC block. A bipolar cuff electrode was placed proximally on the exposed sciatic nerve and a tripolar cuff electrode was placed distally to create the block.

Hook electrodes were also tested for effective generation of a high frequency block. These electrodes were constructed a single length of multistrand silver wire (Medwire AG7/40T, 7 strands, diameter 0.25mm). The wire hooks were inserted under the sciatic nerve and pulled upwards by a 1mm to isolate the sciatic nerve from the surrounding tissue. Silicon oil was also used in this case to further isolate the nerve.

2.5 Experimental procedure

The procedural setup is similar to the one described in Bhadra and Kilgore, 2005. A charge balanced sinusoidal waveform was used for these trials. At each of the nine frequencies – 10kHz, 14kHz, 18kHz, 22kHz, 26kHz, 30kHz, 34kHz, 38kHz and 42kHz – the p-p voltage was varied from 4V to

20V in steps of 2V. Thus, a total of 81 trials were conducted. To determine the optimal proximal stimulation parameters, the maximum current of 1mA was selected on the TDT system and the duration of the stimulus was progressively increased till no further increase in muscle force was observed. The stimulus duration was set at this value for all the trials. For each trial in both the sets, the trial was initiated with proximal stimulation for 10 seconds at 1Hz using the TDT system. At the 10 second time point the high frequency block was initiated and maintained for a period of 10 seconds. At the 20 second time point the block was switched off and the proximal stimulation was continued for 10 seconds.

Evoked muscle force production was measured in the Tibialis Anterior (TA) muscle. The right sciatic nerve (where the macro-sieve was implanted) and its branches were isolated from the sciatic notch to below the point of trifurcation, distal to the popliteal artery. Additionally, TA muscle was exposed by making an incision along the anterior portion of the right leg. The distal tendons of the TA were exposed, isolated and severed as distally as possible from the underlying bone. In order to obtain force measurements, animals were immobilized using a C-clamp to anchor the femoral condyles and distal tendons were attached to thin film load cells (S100, Strain Measurement Devices Inc., Meriden, CT). The load cells transduced evoked muscle force responses to electrical stimulation of the macro-sieve electrode sites as well as silver wire electrodes placed on the sciatic nerve proximal to the MSE assembly.

2.6 Results and discussion

A complete and quickly reversible block was obtained using the tripolar ribbon type cuff electrode. The frequency-voltage characteristic of this block is summarized in Table 2.1

Along with the tripolar cuff electrode the following were also tested for their efficacy in creating a block.

- Bipolar cuff electrode

- Tripolar ring type cuff electrode

No block was observed using either of these electrode configurations. These were tested for the same frequency and voltage ranges – 10 – 30kHz and 4 to 20V. The tripolar ribbon type cuff electrode with the silver epoxy had the same pattern of frequency voltage characteristic as the tripolar ribbon type electrode

Table 2.1: Frequency voltage characteristic of the block obtained with the tripolar ribbon type cuff electrode. B – Block was observed. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block

	p-p Voltage (V)								
Frequency (kHz)	2	4	6	8	10	12	14	16	18
8	EF	EF	EF	EF	EF	EF	EF	EF	EF
10	EF	EF	EF	EF	EF	EF	EF	EF	EF
14	EF	B 2S AF	B 2S AF	B 2S AF	B	B	B	B	B
18	NB 2S AF	PB	B	B	B	B	B	B	B
22	PB	B	B	B	B	B	B	B	B
26	NB	NB 2S AF	PB 2S AF	B	B	B	B	B	B
30	NB	NB 2S AF	NB 3S AF	PB 2S AF	B	B	B	B	B
34	NB	NB	NB 4S AF	PB 3S AF	PB 2S AF	B	B	B	B
38	NB	NB	NB 2S AF	NB	NB	PB 2S AF	PB	EF	B
42	NB	NB	NB	NB	NB 2S AF	PB 2S AF	EF	B	B

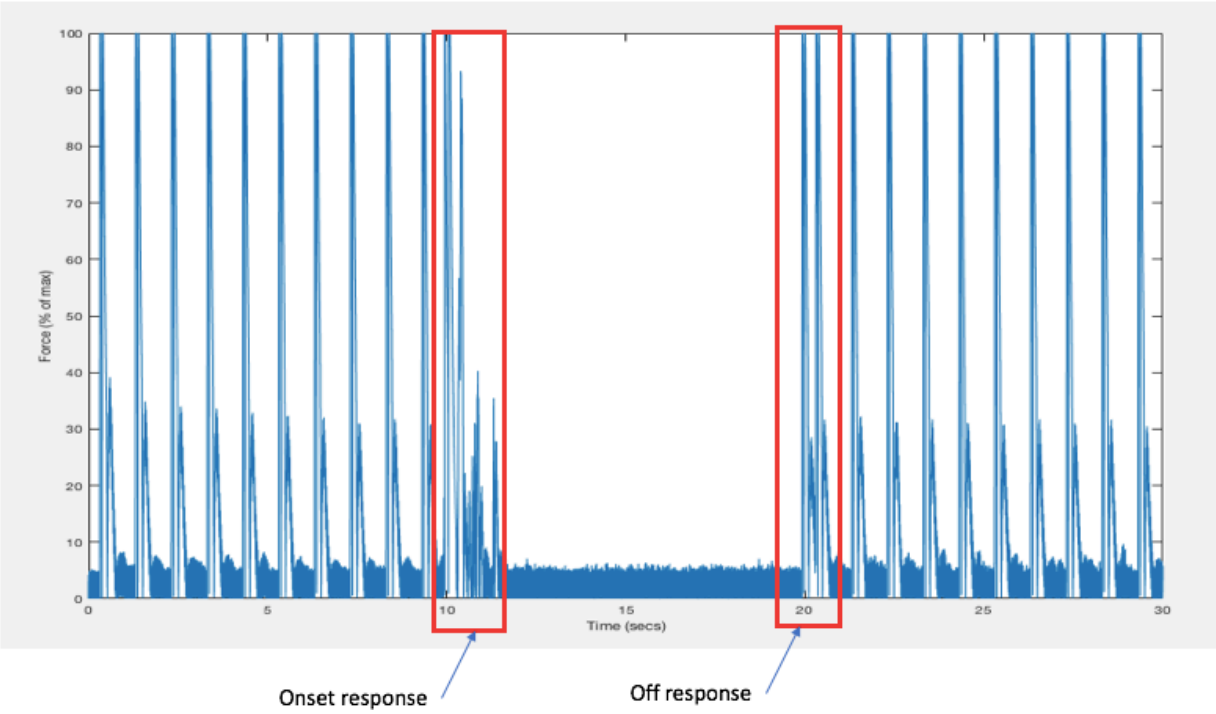


Figure 2.6: Muscle force recordings in tripolar cuff configuration. A complete block was observed. The onset response can be seen at the 10s time point when the HFAC block was switched on. Also, an off response was observed at the 20s time point when the HFAC block was switched off.

Chapter 3

High Frequency Block Using Macro-sieve Electrodes

3.1 Electrode design

3.1.1 Single macro-sieve electrode assembly

A single macro-sieve electrode (MSE) was used for constructing this electrode. The macro-sieve electrode has eight Pt-Ir electrode terminals – four circumferential electrode terminals around the central transit zone and four peripheral electrode terminals on alternate radial spokes. Silicon conduits (AM Systems, #808500 Diameter: Inside 0.078 in., Outside 0.125 in.) of length 2mm were attached to both sides of a single macro-sieve electrode. An Omnetics connector with 8 terminal wires was connected to each of the 8 electrode terminals of the macro-sieve electrode. The solder points were covered with medical grade epoxy for protection from the electrode assembly and insulation from the surrounding medium.

3.1.2 Double macro-sieve electrode 1

Two macro-sieve electrodes were used for constructing this electrode. Two of these MSEs were placed back to back with a thin layer of medical grade adhesive. The gap between the MSEs was measured to be ~0.3mm. 16 channels of an Omnetics 18 pin female connector was soldered to the leads of the electrode terminals. Biomedical silicon tube (AM Systems, #808500 Diameter: Inside 0.078 in., Outside 0.125 in.) was attached to both sides of the double MSE assembly using the same

medical grade adhesive. The solder points were covered with medical grade epoxy for protection from the electrode assembly and insulation from the surrounding medium.

Table 3.1 Impedances of eight sieve assemblies. Overall mean impedance of each channels was 7925 Ohms with a s.d. of 4047 Ohms.

	Sieve Assembly Impedances (Ohms)								
Channel	I	II	III	IV	V	VI	VII	VIII	Mean
Type	b2b	1mm	b2b	1mm	1mm	b2b	1mm	b2b	
1	5832	13098	4893	10217	11067	7299	5579	4070	7757
2	6406	6784	5166	7570	13049	8460	3896	2440	6721
3	5530	6508	5750	11791	15469	5753	4491	2708	7250
4	5813	8684	6140	3458	15744	7519	3820	3279	6807
5	5733	7142	10057	11334	16340	7437	3710	4642	8299
6	5197	7950	7428	15590	17977	6179	4806	3819	8618
7	5524	9020	5987	8191	18995	8088	4863	4332	8125
8	12933	11078	7904	12132	14722	7409	6270	3218	9458
9	7294	12111	5841	2804	16588	12724	6761	7953	9010
10	5206	6463	5623	3137	20600	5936	8229	8781	7997
11	5888	8629	5068	6508	15553	6886	7702	9086	8165
12	4850	4254	6177	8693	18416	8225	7162	8532	8289
13	5145	4385	5942	4157	15170	7090	6913	8641	7180
14	5336	4089	6305	3435	18918	7684	6716	4493	7122
15	5485	5991	5711	4902	11863	8542	5516	7863	6984
16	8028	8382	5177	3461	20286	9652	5712	11393	9011
Mean	6262	7786	6198	7336	16297	7805	5759	5953	7925

3.1.3 Double macro-sieve electrode 2

This electrode was identical in construction to double macro-sieve electrode 1 except that a 1 mm biomedical silicon tubing (AM Systems, #808500 Diameter: Inside 0.078 in., Outside 0.125 in.) was used to create a gap of 1mm between the two macro-sieve electrodes.

3.2 Experimental design

One Lewis rat was implanted with the single macro-sieve electrode assembly. Four Lewis rats underwent a surgical transection of the sciatic nerve followed by microsurgical implantation of double macro-sieve electrode 1 and four more Lewis rats were implanted with double macro-sieve electrode 2.

3.3 Surgical procedure

The Lewis rats were anesthetized by inhalation using 5% Isoflurane for induction and 2% for maintenance. For maintenance, the 2% Isoflurane was progressively reduced to 1.5% during the course of the surgery in order to reduce the detrimental effects of prolonged inhalation of Isoflurane. All surgical procedures were conducted with aseptic techniques. The sciatic nerve was transected approximately 5mm from the trifurcation. Both ends of the transection – proximal and distal – were placed inside the silicone guidance tubes present on either side of the sieve assembly and two diametrically opposite sutures (8-0 Ethilon) were placed on each of the proximal and distal nerve endings to secure it in place inside the silicone guidance tubes. Saline was used to fill up the space inside the sieve assembly before applying the sutures. For six out of the nine implantations, the microwire leads of the macro-sieve assembly were placed in a subcutaneous pocket in the back of the rat. For the other two implantations, the microwires were routed under the proximal musculature. This was done to prevent the bending moment of the microwires to cause it to dislodge the macro-sieve assembly from its position at the transection of the sciatic and thus prevent regeneration of the nerve through the sieve assembly. Before implantation, the Omnetics connector

terminal was wrapped in Parafilm (Parafilm PM996 Wrap) in order to prevent deposition of scar tissue on the connector leads. 5-0 vicryl suture was used to close the muscle and 4-0 nylon suture was used after that to close the skin. Antibiotic ointment was applied externally on the area of the incision to prevent any infection. In some cases, a bitter spray was used to prevent autophagia and disruption of the wound.

At approximately four months after the implantation a non-survival surgery was done to expose the sciatic nerve and distal musculature for assessment of nerve conduction and the efficacy of a high frequency block using the distal MSE. A subcutaneous injection of 3ml saline was given to the rat. Atropine was injected into the muscle to reduce production of saliva during the surgery. The Omnetics connector was exposed from the subcutaneous pocket at the back of the rat and connected to a MS16 stimulus isolator. The MS16 output was controlled using a TDT RX7 processor. A custom circuit was created in RPvsDx software to generate monophasic stimuli at 1Hz to the proximal macro-sieve. A function generator (BK Precision 4003A) was used to deliver the high frequency blocking signal to the distal sieve. In some cases, a distal bipolar cuff electrode was used to deliver a stimulus distal to the macro-sieve assembly.

3.4 Experimental procedures for single macro-sieve electrode assembly

Rat #K001 had been implanted with a single macro-sieve electrode. Three months post implantation the sciatic along with the macro-sieve implantation was exposed for testing. There was good regeneration of the nerve through the macro-sieve as observed from the muscle twitches and EMG recordings. The threshold for observing a muscle twitch was 100 μA at 0.2 ms cathodic stimulus.

The following electrode configurations were tested to determine the availability of a block.

3.4.1 Experimental setup 1

A bipolar cuff electrode was placed proximal to the macro-sieve electrode assembly (Figure 3.1). A Ethilon 4.0 suture was used to tie the cuff electrode externally to ensure maximum surface area of contact of the electrode terminals with the nerve. The terminals of the bipolar cuff electrode were connected to the MS16/RX7 TDT system and used to stimulate the nerve using constant current monophasic cathodic pulses of 1mA at 200us pulse width. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

All 8 electrode terminals of the macro-sieve assembly were connected to a function generator (BK Precision 4003A). The reference for the block signal was placed in the tail of the rat. The function generator was used to give a charge balanced HFAC sinusoidal signal. The following frequencies (in kHz) were tested – 10 to 42KHz in steps of 4kHz. For each of the frequencies the p-p voltage of the sinusoidal waveform was varied from 4 to 20V in steps of 2V (a total of 9x9=81 trials).

No block was observed in any of the frequency voltage combinations, that is muscle twitches as observed directly and on the force sensor readings from the TDT system were of the same amplitude as without the blocking signal (Figure 3.1).

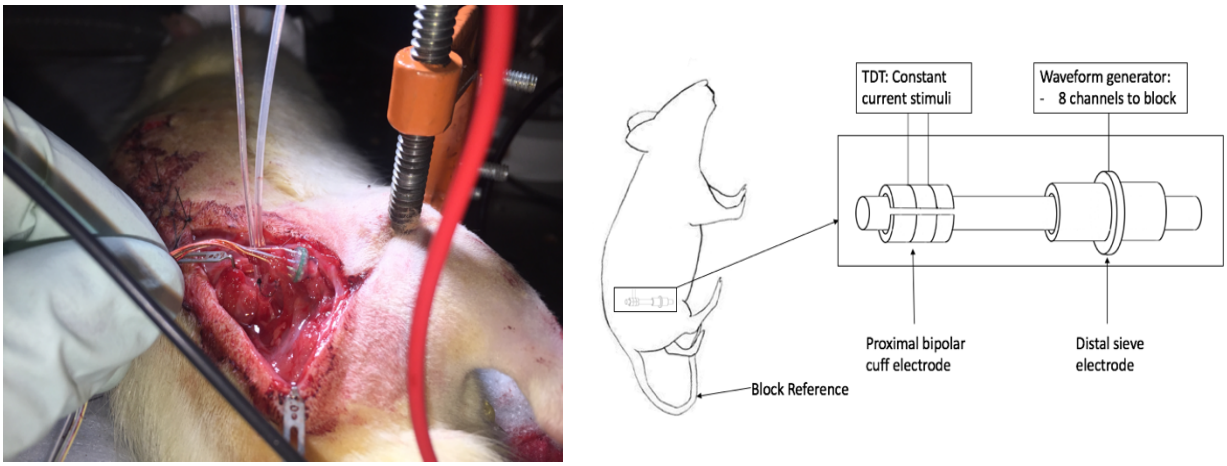


Figure 3.1: Experimental setup 1 (single macro-sieve assembly). Left panel – image of the bipolar cuff connected proximal to the single macro-sieve assembly. Animals were immobilized using anchoring the femoral condyles to a custom force jig. Right panel – schematic showing the proximal bipolar cuff electrode connected to the TDT system for constant current stimuli and the macro-sieve assembly connected to the function generator for the blocking signal. The reference for the block was placed far off in the tail of the rat.

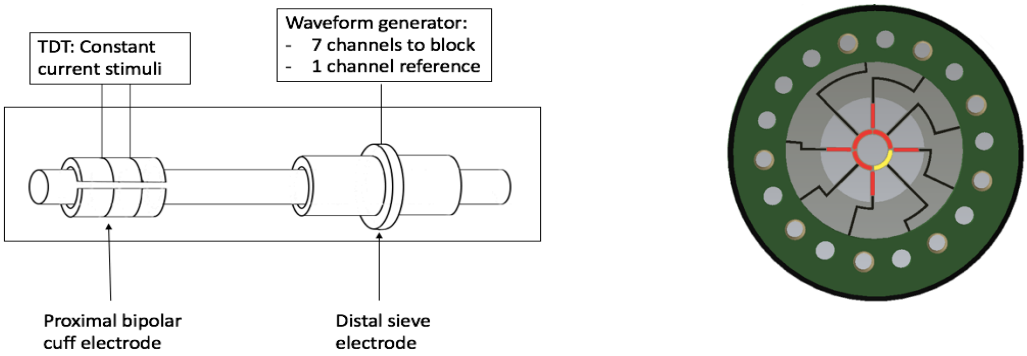


Figure 3.2: Experimental setup 2 (single macro-sieve assembly). Left panel – the setup is similar to experimental setup 1 except for the placing of the block reference (cathode). 7 channels of the macro-sieve were used as the anode of the HFAC block and 1 channel was used as the cathode. Right panel – the red electrode terminals indicate the anode and the yellow terminal indicates the cathode.

3.4.2 Experimental setup 2A

Similar to experimental setup 1 the bipolar cuff electrode was placed proximal to the macro-sieve assembly and was used the deliver the stim using the MS16/RX7 TDT system. 7 of the 8 terminals of the macro-sieve assembly were used to deliver the blocking signal (anode) and the 8th electrode terminal (one of the central four terminals) were used as the cathode for the block signal. The motivation for this setup was to increase the current density of the HFAC blocking signal by creating a local reference as opposed to far off reference in setup 1 where the current density is expected to be much lower. The frequency voltage pairs were same as the ones used in experimental setup 1. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

There was no block observed for any of the frequency voltage combinations.

3.4.3 Experimental setup 2B

The bipolar cuff electrode was again placed proximal to the macro-sieve assembly and was used the deliver the stim using the MS16/RX7 TDT system. For the blocking signal, four of the eight electrode terminals (the four radial terminals – marked in red in Figure 3.3) of the MSE assembly were used as the anode and the center four terminals (marked in yellow in the MSE diagram in Figure 3.3) of the MSE assembly were used as the cathode. The frequency voltage pairs were same as the ones used above. Similar to configuration 2A this configuration also enabled a higher current density for the blocking signal. In addition, the current flow was more symmetric across the cross section of the nerve and was expected to create a more uniform block for all the axons in the nerve.

There was no block observed for any of the frequency voltage combinations.

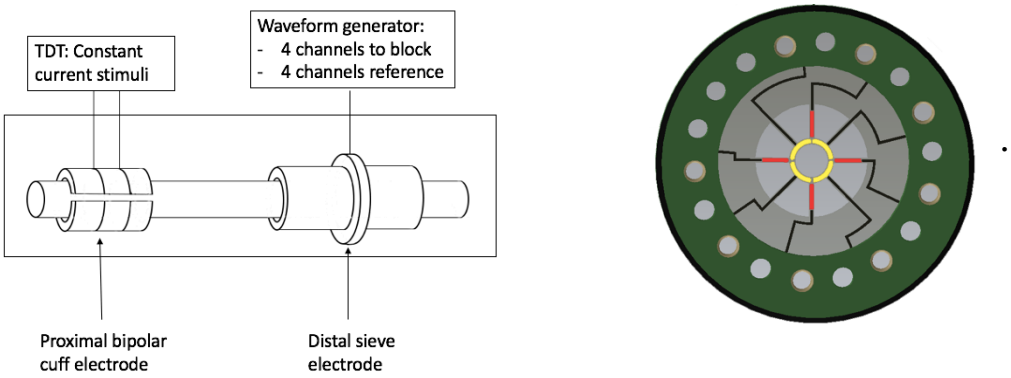


Figure 3.3: Experimental setup 2B (single macro-sieve assembly). Left panel – Experimental setup similar to setup 2A with the proximal bipolar electrode connected to the TDT system and the macro-sieve connected to the function generator. Right panel – four radial electrode terminals (in red) connected to the anode of the function generator and the four central electrode terminals (in yellow) connected to the cathode.

3.4.4 Experimental setup 3

As in experimental setup 2, the bipolar cuff electrode was placed proximal to the macro-sieve assembly and was used the deliver the stim using the MS16/RX7 TDT system. The blocking signal was delivered through the function generator. All 8 channels of the macro-sieve assembly were used as the blocking signal anode. In addition, a monopolar ring type cuff electrode (1 Pt-Ir wire across the circumference of the silicone tube) was placed distal to the macro-sieve electrode and was used the blocking cathode (Figure 3.4). The distance between the macro-sieve electrode and the monopolar cuff electrode was 4 mm from the center of the MSE assembly to the center of the monopolar cuff electrode. The objective of this configuration was to increase the length of the nerve which is affected by the HFAC blocking signal. The frequency voltage pairs were same as the ones used above in the other configurations. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

There was no block observed for any of the frequency voltage combinations.

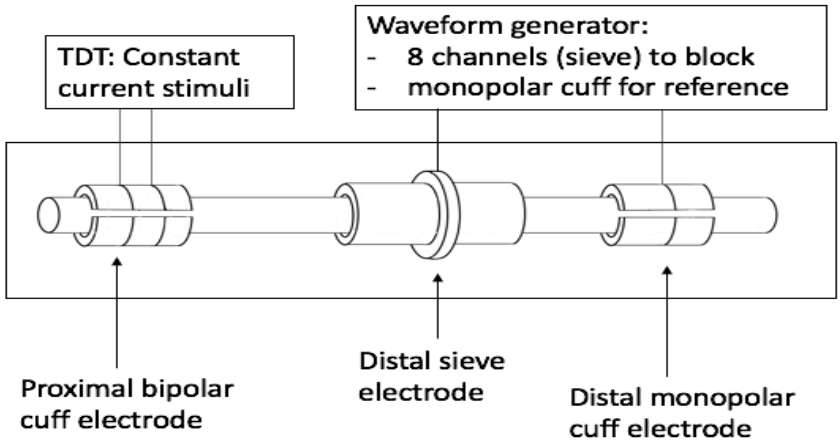


Figure 3.4: Experimental setup 3 (single macro-sieve assembly). Proximal bipolar cuff electrode for stimulation and a hybrid bipolar setup where all eight channels of the sieve electrode were used for the HFAC block and the distal monopolar cuff was used as a reference.

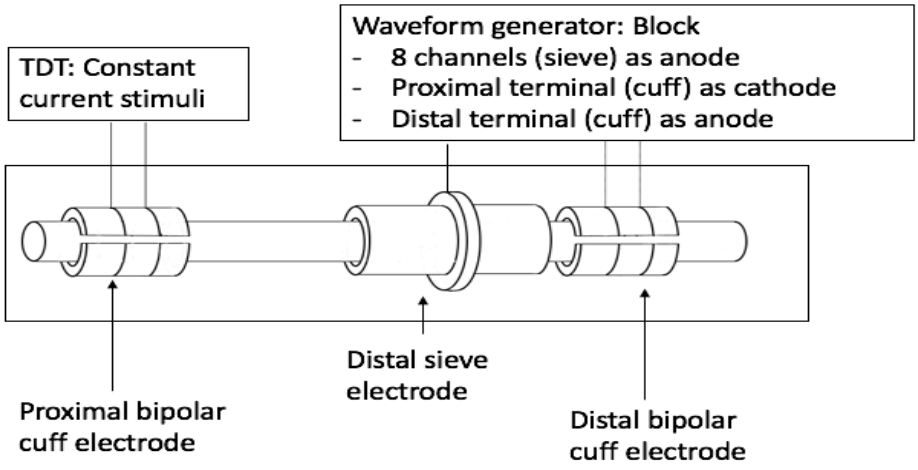


Figure 3.5: Experimental setup 4 (single macro-sieve assembly). Proximal bipolar cuff electrode for stimulation and a hybrid tripolar setup where all the eight electrode terminals of the sieve and the distal electrode terminal of the distal bipolar cuff electrode are used as the anode and the proximal electrode terminal of the distal bipolar cuff is used as the cathode for the HFAC block.

3.4.5 Experimental setup 4

The bipolar cuff electrode was placed proximal to the macro-sieve assembly and was used the deliver the stim using the MS16/RX7 TDT system. The blocking signal was delivered through the function generator. In addition, a bipolar ring type cuff electrode was placed distal to the macro-sieve electrode. All 8 channels of the macro-sieve electrode and the distal terminal of the distal bipolar cuff electrode was used as the anode for the blocking signal. The proximal terminal of the distal bipolar cuff electrode was used as the cathode for the blocking signal (Figure 3.5). This effectively created a hybrid tripolar electrode utilizing the all 8 channels of the macro-sieve assembly and the two terminals of the distal bipolar cuff electrode. The objective of this configuration was to replicate as closely as possible the tripolar cuff electrode configuration that was used in naïve rats to affect a nerve block. The frequency voltage pairs were same as the ones used above in the other configurations. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

There was no block observed for any of the frequency voltage combinations.

3.4.6 Experimental setup 5

The bipolar cuff electrode was placed proximal to the macro-sieve assembly and was used the deliver the stim using the MS16/RX7 TDT system. The blocking signal was delivered through the function generator. A tripolar ring type cuff electrode was distal to the macro-sieve assembly. The central electrode terminal was used as the cathode for the blocking signal and the two flanking electrode terminals were used as the anodes (Figure 3.6). The objective of this configuration was to determine if regenerated axons could be blocked using the same frequency-voltage combinations as the non-regenerated nerve. The frequency voltage pairs were same as the ones used above in the other configurations.

There was no block observed for any of the frequency voltage combinations.

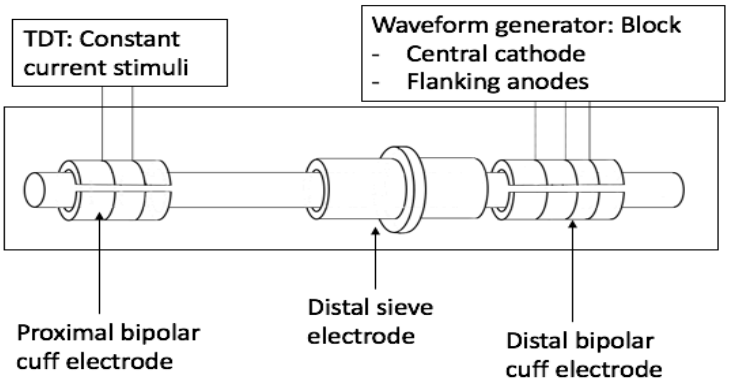


Figure 3.6: Experimental setup 5 (single macro-sieve assembly). Proximal bipolar cuff electrode for stimulation and a tripolar ring type cuff electrode was used for the HFAC block distally.

3.5 Experimental procedures for dual macro-sieve electrode assemblies

3.5.1 Experimental setup 1

One channel in the proximal sieve was used to send the constant current stimulus using the MS16 / RX7 TDT system. The reference for the stimulus signal was placed far off in the tail of the rat. The blocking signal was delivered through the function generator to the distal macro-sieve electrode. All 8 channels of the macro-sieve electrode were used as the anode for the blocking signal and a far-off cathode was placed in the body of the rat in the lower back. Each the eight channels in the proximal macro-sieve electrode were stimulated one by one. For the blocking signal frequencies in the range of 10 to 42 kHz in steps of 4kHz were tested. For each of these frequencies p-p voltages in the range of 4 to 20V in steps of 2V were tested. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

3.5.2 Experimental setup 2A

One channel in the proximal sieve was used to send the constant current stimulus using the MS16 / RX7 TDT system. The reference for the stimulus signal was placed far off in the tail of the rat. The blocking signal was delivered through the function generator to the distal macro-sieve electrode. Seven channels of the macro-sieve electrode were used as the anode for the blocking signal and 1 channel was used as the cathode. For the blocking signal frequencies in the range of 10 to 42 kHz in steps of 4kHz were tested. For each of these frequencies p-p voltages in the range of 2 to 20V in steps of 2V were tested. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

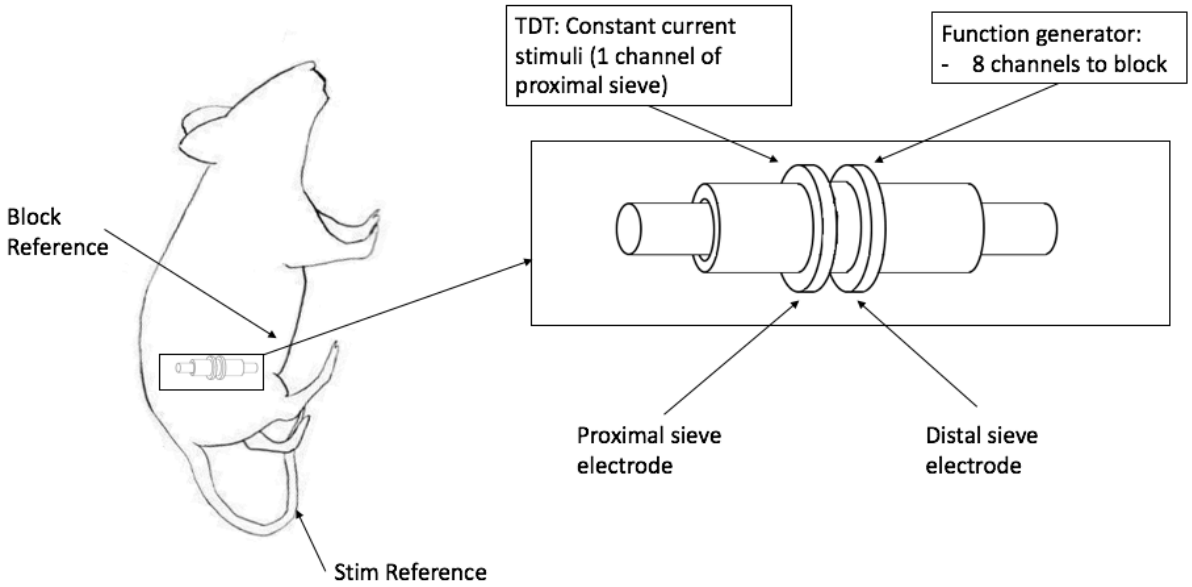


Figure 3.7: Experimental setup 1 (dual macro-sieve assembly). One channel of the proximal electrode was used to deliver the stimulus using the TDT system. The reference was placed far off in the tail. All 8 channels of the distal macro-sieve were used to deliver the HFAC blocking signal and the reference was placed far off in the body of the rat.

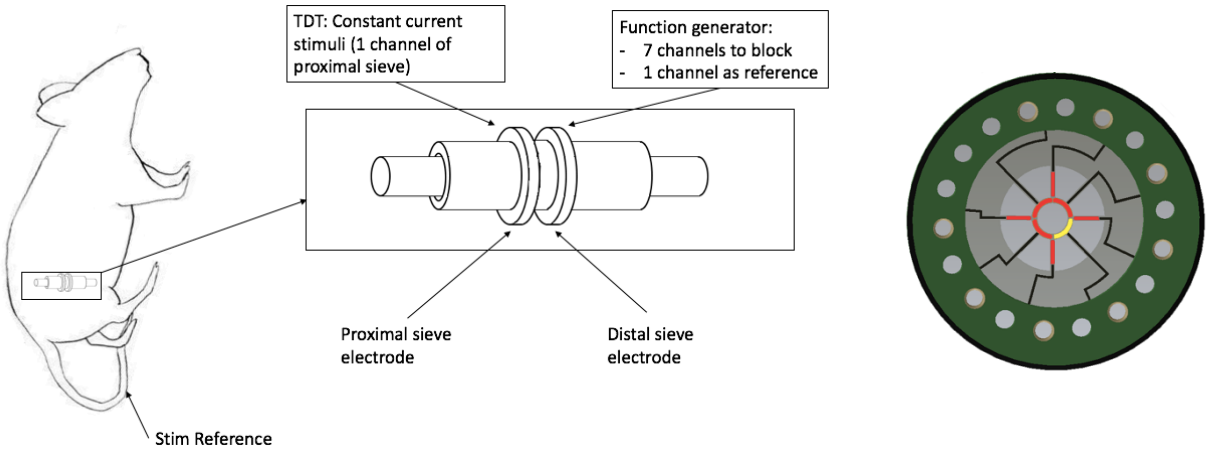


Figure 3.8: Experimental setup 2A (dual macro-sieve assembly). The HFAC blocking signal is delivered to seven of the eight channels of the distal macro-sieve assembly and 1 channel is used as the reference for the block. The stimulus delivery is same as setup 1 above.

3.5.3 Experimental setup 2B

Similar to configuration 2A, one channel in the proximal sieve was used to send the constant current stimulus using the MS16 / RX7 TDT system. The reference for the stimulus signal was placed far off in the tail of the rat. The blocking signal was delivered through the function generator to the distal macro-sieve electrode. The four radial electrode terminals (radial) of the macro-sieve electrode were used as the anode for the blocking signal and 4 channels (center) were used as the cathode. The frequency and voltage values tested were the same as other configurations. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

3.5.4 Experimental setup 2C

Similar to configuration 2B, one channel in the proximal sieve was used to send the constant current stimulus using the MS16 / RX7 TDT system. However, 2 adjacent channels in the proximal macro-sieve were used as the reference. For the blocking signal, four of the eight electrode terminals (the four radial terminals – marked in red in Figure 3.10) of the MSE assembly were used as the anode and the center four terminals (marked in yellow in the MSE diagram in Figure 3.10) of the MSE assembly were used as the cathode. The frequency and voltage values tested were the same as other configurations. The setup for muscle force measurement was identical to the one used for the cuff electrodes (section 2.4).

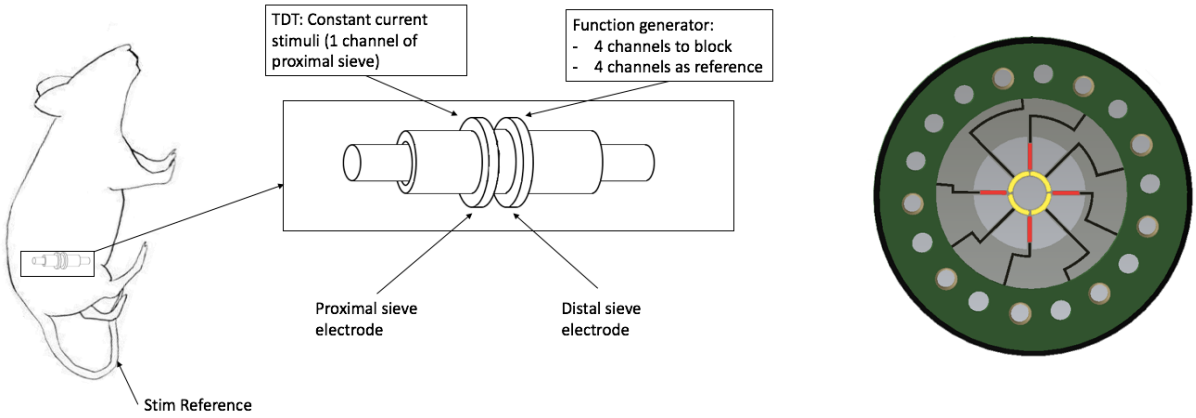


Figure 3.9: Experimental setup 2B (dual macro-sieve assembly): Similar to setup 2A. However, in this case th4 radial channels were used as the HFAC blocking signal and the central circumferential channels were used as the reference.

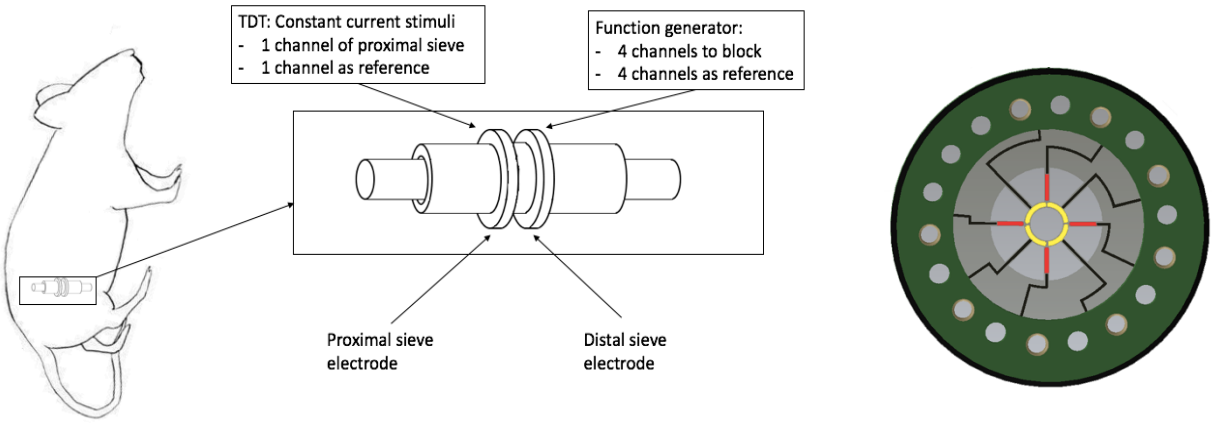


Figure 3.10: Experimental setup 2C (dual macro-sieve assembly). 1 channel of the proximal sieve is used to deliver the stimulus. Adjacent channels on the proximal stim are used as reference. For HFAC block four radial channels are used to deliver the signal and 4 as reference

3.5.5 Experimental setup 3

A monopolar cuff electrode was place distal to the double macro-sieve assembly. The electrode terminal of the monopolar cuff electrode was designed as a ribbon type using Pt-Ir wires woven into a silicon tube. This was done to ensure maximum surface area of contact with the nerve.
As before, one radial electrode terminal in the proximal sieve was used to send the constant current stimulus using the MS16 / RX7 TDT system and two adjacent electrode terminals in the proximal macro-sieve were used as the reference. The function generator was used to deliver the blocking signal. All 8 channels of the distal macro-sieve electrode were used as the anode and the distal monopolar cuff electrode was used as the cathode. The frequency and voltage values tested were the same as other configurations

3.5.5 Experimental setup 4

A bipolar cuff electrode was placed proximal to the double macro-sieve assembly and used to deliver the stimulus using the MS16 / RX7 TDT system. All 8 channels of the proximal macro-sieve electrode were used as the anode for the block signal and all 8 channels of the distal sieve were used as a cathode. The motivation for this configuration was to create a high current density in a small section of the nerve to enable a block. The frequency and voltage values tested were the same as other configurations

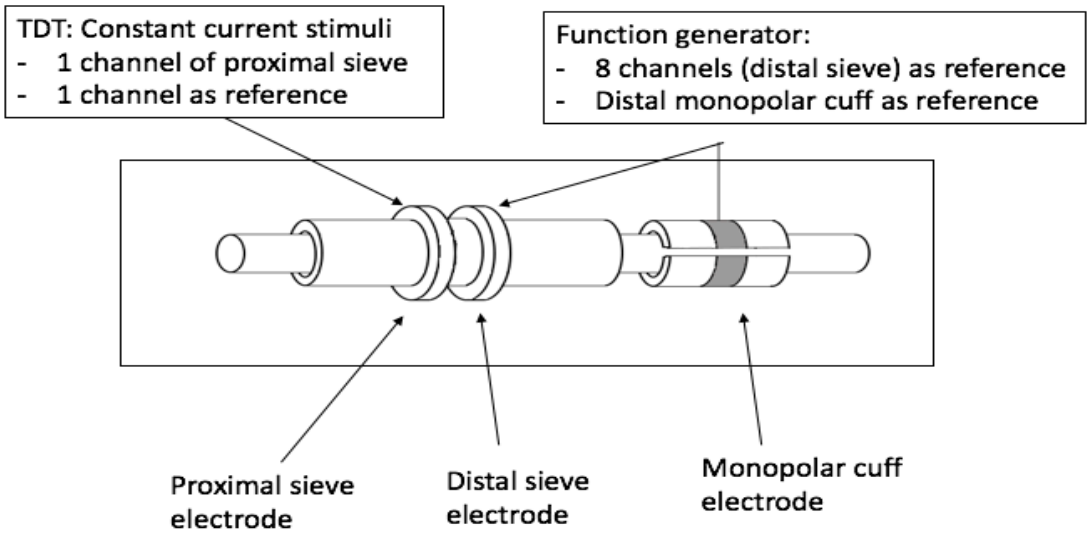


Figure 3.11: Experimental setup 3 (dual macro-sieve assembly). One channel of proximal macro-sieve electrode is used to deliver the stimulus and the adjacent channel as the reference. A hybrid bipolar electrode is created by using the 8 channels of the distal macro-sieve as the anode for the HFAC block and a distal monopolar cuff type electrode is used as the cathode.

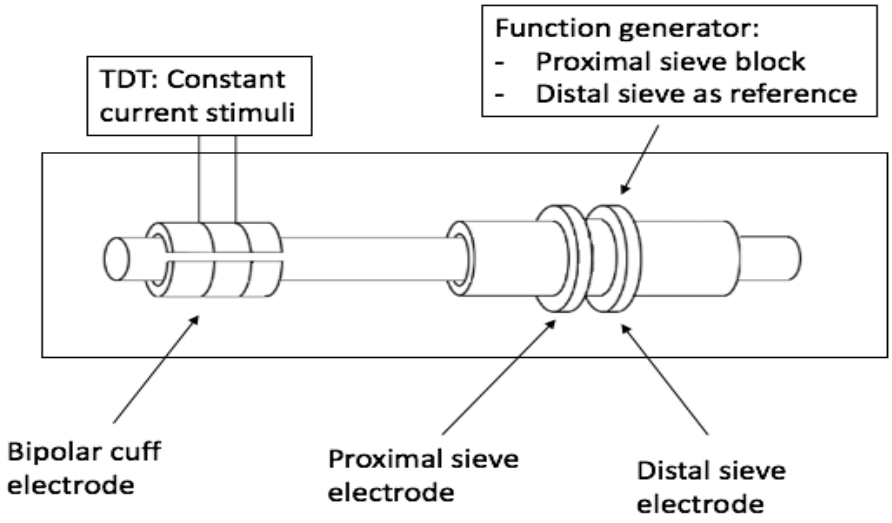


Figure 3.12: Experimental setup 4 (dual macro-sieve assembly). Proximal stim delivered through a bipolar ring type cuff electrode. All eight channels of proximal macro-sieve electrode used as anode for the HFAC blocking signal and the distal macro-sieve used as the cathode.

3.6 Results (dual macro-sieve assembly)

3.6.1 Rat #025

For the first animal (#025), the macro-sieve assembly was observed to be bulging out and significant muscle atrophy was observed in the right hind leg. Before exposing the sciatic a low frequency (1Hz) stim signal was delivered to the proximal and distal electrode sites sequentially to check if the sciatic nerve had regenerated through the dual macro-sieve assembly. No muscle twitches were observed on stimulating, in any of the electrode sites. On making an incision through the skin and musculature at the site of the double macro-sieve implant, it was evident that there was no regeneration through the macro-sieve assembly. The macro-sieve assembly was found in a subcutaneous pocket. The proximal end of the sciatic was embedded deeper in the musculature but was not connected to the distal end.

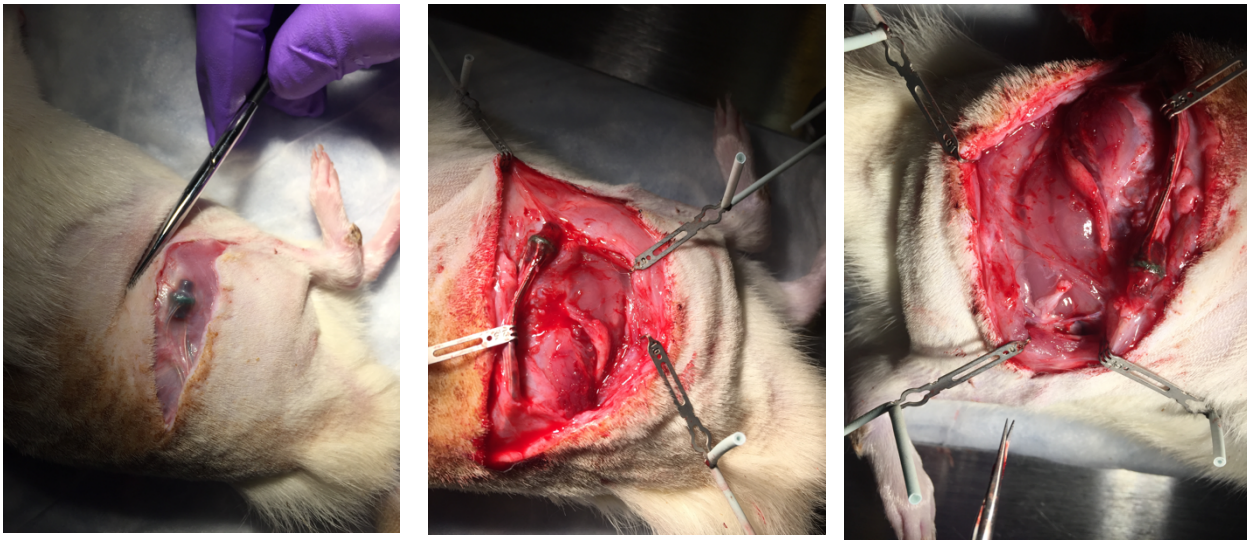


Figure 3.13: Rat #025. No regeneration of the sciatic nerve through the dual macro-sieve assembly

Analysis: The double macro-sieve required 16 microwire leads (8 for each macro-sieve). These microwires are placed so that they bend upwards from the macro-sieve assembly. The microwires exit the musculature and are bent again so that lie subcutaneously along the back of the animal. Because of these bends and the combined bending moment of the of 16 microwires, it is presumed that the macro-sieve assembly was pushed up to a subcutaneous position causing the sciatic nerve to tear away from the sutures with the silicone conduit at the both the proximal and distal ends of the nerve. Thus, no regeneration could happen through the macro-sieve assembly. Based on this observation subsequent double macro-sieve implants were performed using the technique described earlier (section 3.3), where the microwires arising from the macro-sieve assembly were passed underneath the proximal musculature. This would effectively reduce the force exerted by the bending moment of the microwires and thus keep the macro-sieve assembly in its intended position. As an additional measure to prevent dislodgement of the macro-sieve assembly, and additional suture was used to tie the macro-sieve assembly to the underlying musculature.

3.6.2 Rat #7066

For the second animal (#7066), there was no bulging out of the macro-sieve assembly observed. There was some muscle atrophy observed in the right hind leg where the double macro-sieve assembly had been implanted. Before exposing the sciatic nerve a low frequency (1Hz) stim signal was delivered to the proximal and distal macro-sieve electrodes sequentially to check if the sciatic nerve had regenerated through the dual macro-sieve assembly. Muscle twitches were observed on stimulating in all the electrode sites. However, the threshold for observing muscle twitches were significantly higher than those reported earlier (with single macro-sieve implants). Observed threshold was 500uA at 0.2ms duration.

Table 3.2: Animal #7066 – Experimental setup 1, no block was observed at the various frequencies and amplitudes tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block.

	p-p Voltage (V)								
Frequency (kHz)	4	6	8	10	12	14	16	18	20
10	NB	NB	NB	NB (3s AF)	NB (5s AF)	EF	EF	EF	EF
14	NB	NB	NB	NB (2s AF)	NB (4s AF)	NB (5s AF)	EF	EF	EF
18	NB	NB	NB	NB	NB (1s AF)	NB (3s AF)	NB (4s AF)	NB (5s AF)	EF
22	NB	NB	NB	NB	NB	NB (1s AF)	NB (2s AF)	NB (3s AF)	EF
26	NB	NB	NB	NB	NB	NB	NB	NB	NB (1s AF)
30	NB	NB	NB	NB	NB	NB	NB	NB	NB
34	NB	NB	NB	NB	NB	NB	NB	NB	NB
38	NB	NB	NB	NB	NB	NB	NB	NB	NB
42	NB	NB	NB	NB	NB	NB	NB	NB	NB

An onset response was observed when the high frequency blocking signal was turned on in all cases. In some cases, there was some asynchronous firing post initiation of the block and in some other cases there excessive firing (muscle twitches) post initiation of the block. In none of the frequency amplitude combinations was a block observed.

Table 3.3: Rat #7066 - Experimental setup 2. Local references for stimulation and HFAC blocking signal. no block was observed at the various frequencies and amplitudes tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block.

	p-p Voltage (V)								
Frequency (kHz)	4	6	8	10	12	14	16	18	20
10	NB	EF	EF	EF	EF	EF	EF	EF	EF
14	NB	NB (1s AF)	EF	EF	EF	EF	EF	EF	EF
18	NB	NB	NB (3s AF)	EF	EF	EF	EF	EF	EF
22	NB	NB	NB	NB (4s AF)	EF	EF	EF	EF	EF
26	NB	NB	NB	NB (1s AF)	NB (3s AF)	NB (5s AF)	EF	EF	EF
30	NB	NB	NB	NB	NB	NB	NB	NB	NB
34	NB	NB	NB	NB	NB	NB	NB	NB	NB
38	NB	NB	NB	NB	NB	NB	NB	NB	NB
42	NB	NB	NB	NB	NB	NB	NB	NB	NB

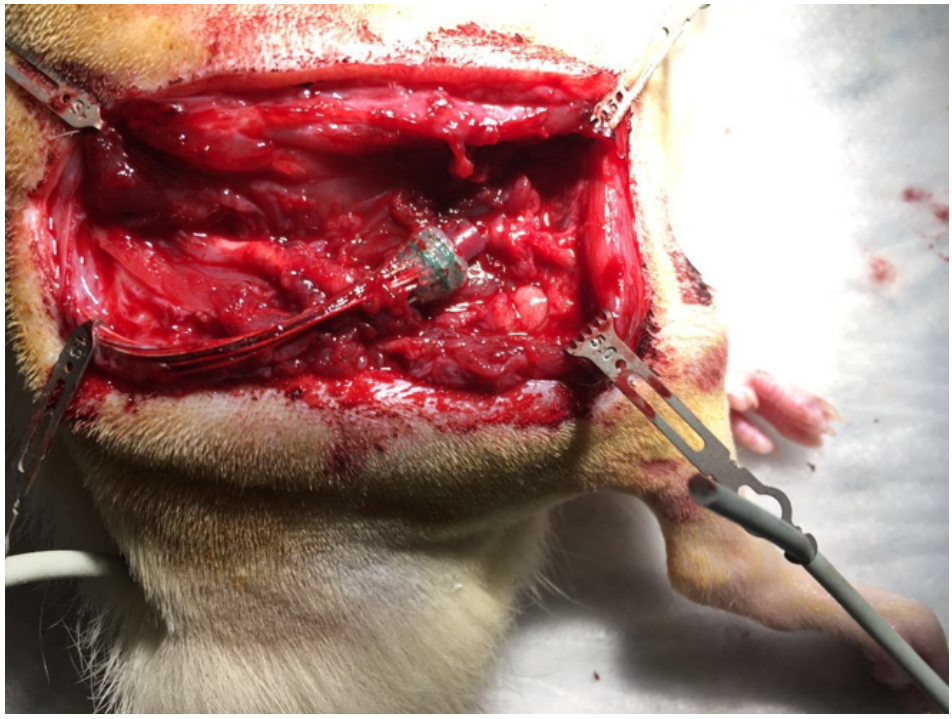


Figure 3.14: Rat #7066: Partial regeneration of the sciatic nerve through the dual macro-sieve assembly.

Experimental setup 3 and 4: On exposing the sciatic at the site of the implant, it was observed that the macro-sieve assembly was located relatively much more distally as compared to other macro-sieve implants, close to the trifurcation. The proximal end of the sciatic was in close contact with the proximal silicone conduit of the macro-sieve assembly. However, there was significant scar tissue around the point of contact. The distal end of the sciatic was much smaller in diameter as compared to the proximal end of the sciatic. In addition to the regeneration of the sciatic through macro-sieve assembly, the proximal end of the sciatic branched off at the point of contact with the macro-sieve assembly and formed a neuromuscular junction with the underlying musculature at the site of the implant thus bypassing the macro-sieve assembly. Therefore, experimental setups 3 and 4 were not used since the branch of the sciatic was directly innervating the muscle, rendering the blocking macro-sieve electrode unusable.

The sciatic nerve was transected as proximally as possible and at the junction of the branch of the sciatic with the underlying the musculature. The section of the sciatic along with the macro-sieve assembly was explanted and immersed in glutaraldehyde for histological analysis.

3.6.2 Rat #7067

The Omnetics connector was exposed from the subcutaneous pocket where it was buried. On stimulating with a constant current cathodic stimulus using the MS16/RX7 TDT system the observed threshold current for muscle twitches was observed to be 50uA which confirmed regeneration of the nerve through the dual macro-sieve assembly.

The following electrode configurations were tested to check the availability of a complete nerve block.

- No block was observed in experimental setup 1 (Table 3.4)
- No block was observed in experimental setup 2 (Table 3.5)

For experimental setup 3,4,5 & 6 - to enable this configuration the sciatic nerve of the rat along with the implanted double macro-sieve assembly was exposed. It was observed that a branch of the sciatic nerve originating from the proximal part of the nerve was bypassing the double macro-sieve assembly and directly innervating the distal musculature as shown in Figure 3.9 However this branch a was a minor one as compared to the major part of the nerve which had regenerated through the double macro-sieve assembly. The minor branch bypassing the double macro-sieve assembly was transected.

Table 3.4: Rat #7067 – Experimental setup 1. No block was observed for the range of voltages and frequencies tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block.

	p-p Voltage (V)								
Frequency (kHz)	4	6	8	10	12	14	16	18	20
10	EF	EF	EF	EF	EF	EF	EF	EF	EF
14	NB 5s AF	EF	EF	EF	EF	EF	EF	EF	EF
18	NB 5s AF	EF	EF	EF	EF	EF	EF	EF	EF
22	NB	NB 5s AF	EF	EF	EF	EF	EF	EF	EF
26	NB	NB	NB 4s AF	EF	EF	EF	EF	EF	EF
30	NB	NB 2s AF	NB 2s AF	NB 2s AF	NB 4s AF	EF	EF	EF	EF
34	NB	NB	NB	NB 4s AF	NB 4s AF	EF	EF	EF	EF
38	NB	NB	NB	NB 2s AF	NB 4s AF	NB 4s AF	NB 4s AF	EF	EF
42	NB	NB	NB	NB	NB 2s AF	NB 3s AF	NB 4s AF	EF	EF

Table 3.5: Rat #7067 – Experimental setup 2. No block was observed for the range of voltages and frequencies tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block

	p-p Voltage (V)									
Frequency (kHz)	2	4	6	8	10	12	14	16	18	20
10	NB	NB	NB	NB 4s AF	EF	EF	EF	EF	EF	EF
14	NB	NB	NB	NB	NB 4s AF	EF	EF	EF	EF	EF
18	NB	NB	NB	NB	NB	NB 3s AF	EF	EF	EF	EF
22	NB	NB	NB	NB	NB	NB	NB 3s AF	EF	EF	EF
26	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB 3s AF
30	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB 3s AF
34	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB 3s AF
38	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
42	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB

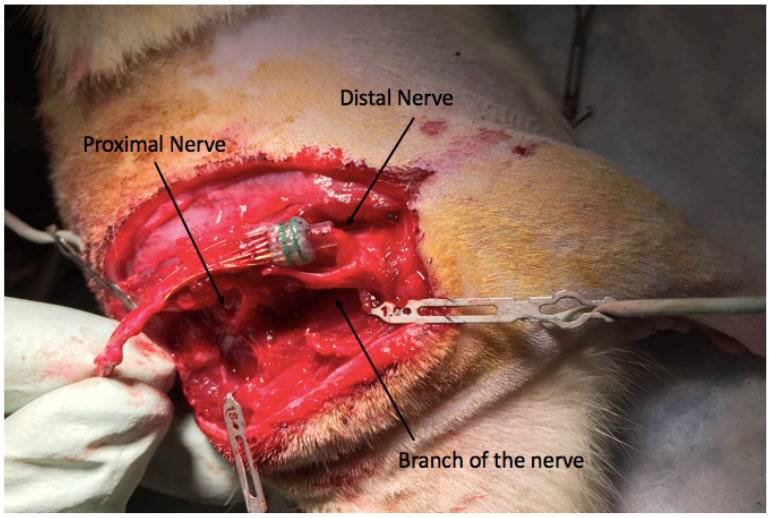


Figure 3.15: Rat #7067 - Branching of the proximal end.

3.6.2 Rat #091

The following results were obtained

- Experimental setup 1: No block was observed (Table 3.6)
- Experimental setup 2: No block was observed (Table 3.7)
- Experimental setup 3: No block was observed (Table 3.8)
- Experimental setup 4: Block was observed (Table 3.9)
- Experimental setup 5: No block was observed (Table 3.10)
- Experimental setup 6: No block was observed (Table 3.11)

Table 3.6: Rat #091 – Experimental setup 1. No block was observed for the range of voltages and frequencies tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block.

	p-p Voltage (V)								
Frequency (kHz)	4	6	8	10	12	14	16	18	20
10	NB	EF	EF	EF	EF	EF	EF	EF	EF
14	NB	NB 2s AF	EF	EF	EF	EF	EF	EF	EF
18	NB	NB	NB 2s AF	EF	EF	EF	EF	EF	EF
22	NB	NB	NB	NB 3s AF	EF	EF	EF	EF	EF
26	NB	NB	NB	NB 2s AF	NB 4s AF	EF	EF	EF	EF
30	NB	NB	NB	NB	NB 2s AF	NB 3s AF	EF	EF	EF
34	NB	NB	NB	NB	NB	NB	NB 2s AF	NB 3s AF	NB 4s AF
38	NB	NB	NB	NB	NB	NB	NB	NB	NB 2s AF
42	NB	NB	NB	NB	NB	NB	NB	NB	NB 2s AF

Table 3.7: Rat #091 – Experimental setup 2. No block was observed for the range of voltages and frequencies tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block.

	p-p Voltage (V)								
Frequency (kHz)	4	6	8	10	12	14	16	18	20
10	NB	NB 2s AF	NB	EF	EF	EF	EF	EF	EF
14	NB	NB	NB	NB	NB 2s AF	NB 3s AF	EF	EF	EF
18	NB	NB	NB	NB	NB	NB	NB	EF	EF
22	NB	NB	NB	NB	NB	NB	NB	NB	EF
26	NB	NB	NB	NB	NB	NB	NB	NB	NB 2s AF
30	NB	NB	NB	NB	NB	NB	NB	NB	NB 2s AF
34	NB	NB	NB	NB	NB	NB	NB	NB	NB
38	NB	NB	NB	NB	NB	NB	NB	NB	NB
42	NB	NB	NB	NB	NB	NB	NB	NB	NB

Table 3.8: Rat #091 – Experimental setup 3. No block was observed for the range of voltages and frequencies tested. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block.

	p-p Voltage (V)								
Frequency (kHz)	4	6	8	10	12	14	16	18	20
10	EF	EF	EF	EF	EF	EF	EF	EF	EF
14	EF	EF	EF	EF	EF	EF	EF	EF	EF
18	EF	EF	EF	EF	EF	EF	EF	EF	EF
22	NB	EF	EF	EF	EF	EF	EF	EF	EF
26	NB	EF	EF	EF	EF	EF	EF	EF	EF
30	NB	NB 2s AF	EF	EF	EF	EF	EF	EF	EF
34	NB	NB	NB	EF	EF	EF	EF	EF	EF
38	NB	NB	NB 2s AF	NB 2s AF	EF	EF	EF	EF	EF
42	NB	NB	NB	NB 2s AF	NB 2s AF	NB 4s AF	EF	EF	EF

Table 3.9: Rat #091 – Experimental setup 4. Block was observed. B – Block was observed. NB – no block observed, (1s AF) – 1 second of asynchronous firing post initiation of block, EF – excessive asynchronous firing post initiation of block

	p-p Voltage (V)									
Frequency (kHz)	2	4	6	8	10	12	14	16	18	20
10	NB 2s AF	EF	EF	EF	EF	EF	EF	EF	EF	EF
14	NB	NB 4s AF	NB 4s AF	PB	PB	B	B	B	B	B
18	NB	NB	NB	NB	NB	PB	PB	B	B	B
22	NB	NB	NB	NB	NB	PB	PB	PB	B	B
26	NB	NB	NB	NB	NB	PB	PB	PB	B	B
30	NB	NB	NB	NB	NB	NB	PB	PB	PB	PB
34	NB	NB	NB	NB	NB	NB	NB	PB	PB	PB
38	NB	NB	NB	NB	NB	NB	NB	PB	PB	PB
42	NB	NB	NB	NB	NB	NB	NB	NB	NB	PB

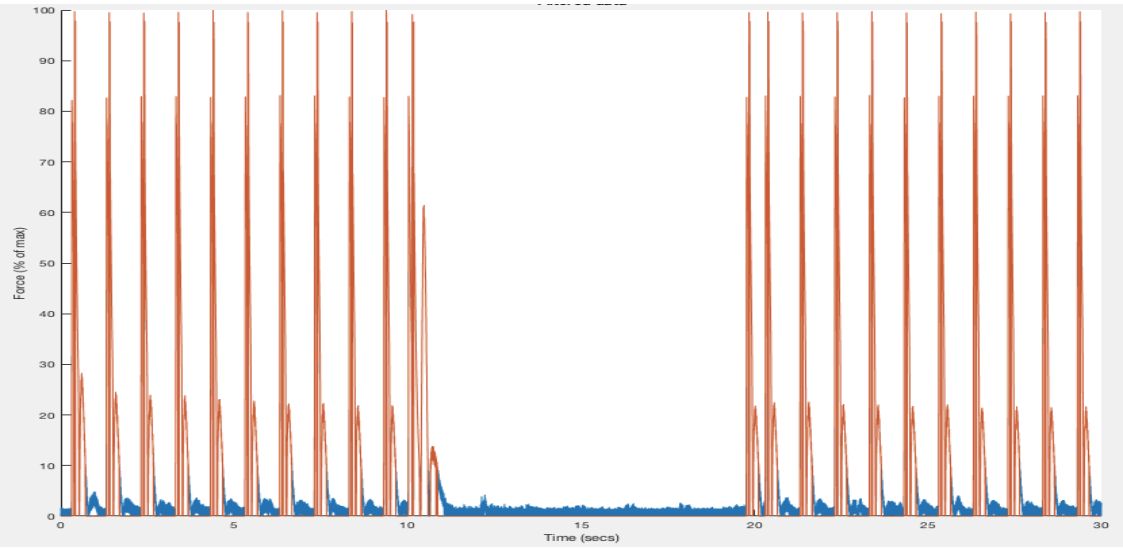


Figure 3.16: Muscle force data showing a complete block for Rat #091. Blocking signal frequency of 18KHz**,** 20V p-p. The data was filtered using a low pass filter with a cutoff frequency of 300Hz. The standard deviation of the baseline (without stimulus) data was determined. The red lines indicate the time points at which the signal exceeded the baseline by more than one standard deviations thus indicating a CAP. When the HFAC blocking signal was switched on, after the initial onset response no further CAPs were observed during the time the HFAC block was on (no red lines in the block period), thus indicating a complete block.

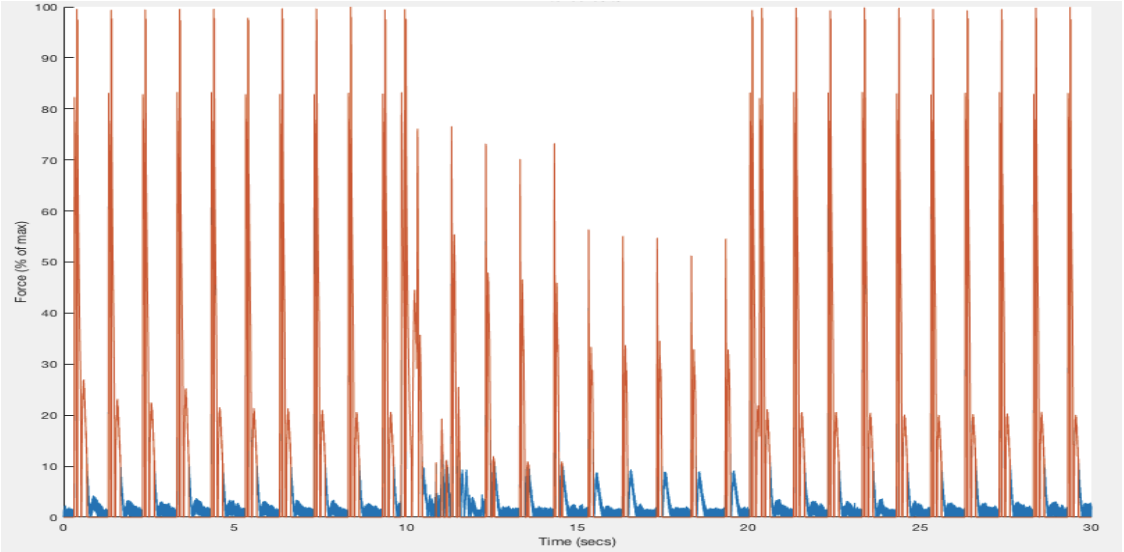


Figure 3.17: Muscle force data showing a partial block for Rat #091. Blocking signal frequency of 18KHz**,** 12V p-p. The same procedure (as described in Figure 3.11) was used to determine the presence of an action potential. In the 10 to 20s timeframe the muscle force amplitude is observed to be less than the maximum observed in absence of the blocking signal.

3.7 Discussion

For experimental setups 1 and 2, one possible reason for the absence of any observable block is that the HFAC block requires a minimum length of the nerve to affected by the blocking signal. In these two setups, the current flow was effectively across a single cross-sectional plane of the nerve.

The HFAC block was only observed in the experimental setup where both the macro-sieves in the dual sieve assembly were used for the block. A high frequency voltage controlled function generator had been used to create the blocking signal. Also, in this particular setup the distance between the anode and cathode was 1mm with all the eight channels of the macro-sieve effectively in parallel. Both these factors – 1mm gap and parallel impedances – cause to reduce the impedance and thus a relatively higher current was passed through the nerve.

For experimental setup 4 where a monopolar cuff electrode was used distally to act as the cathode for the HFAC block, it might be possible to get a block if a constant current source is used to give a much higher current. Although based on the observed frequency–voltage characteristic there is excessive firing at higher voltages and higher frequencies will be necessary to enable the block.

Chapter 4

Modelling the High Frequency Block

4.1 Model development

A CAD model of the macro-sieve electrode was created in Solidworks based on a high-resolution photo of the macro-sieve electrode so as to create an accurate representation of the dimensions of each electrode terminal (Figure 3.1)

This Solidworks drawing was imported into Comsol to create a finite element model of the potential in the nerve. The nerve was modelled as a uniform cylinder and the conduit of the double macro-sieve unit was modelled as a hollow cylinder. A surrounding sphere of saline was created as a model approximation of the conductive tissue around the nerve. Table 4.1 provide the specifications of the model.

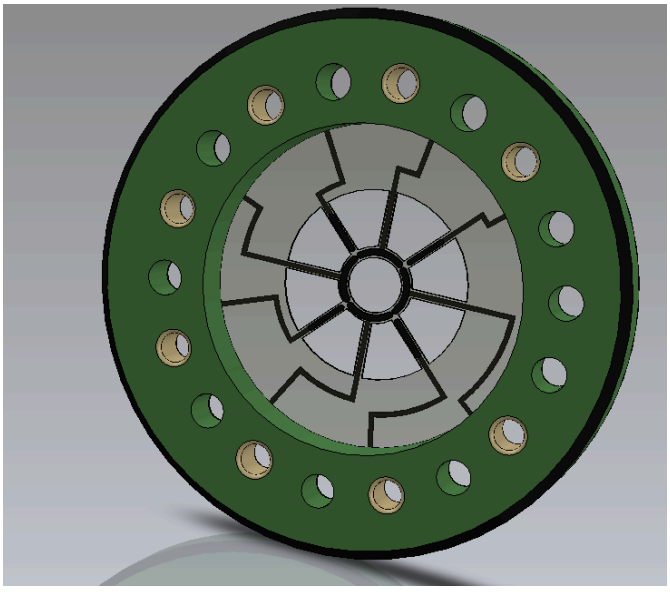


Figure 4.1: Solidworks CAD model of the macro-sieve electrode

Table 4.1: Parameters for Comsol model of dual macro-sieve assembly

Model part	Dimensions	Conductivity
Nerve	Length: 9 cm Radius: 1 mm	Anisotropic X: 0.083 S/m Y: 0.083 S/m Z: 0.571 S/m (along the length of the nerve)
Conduit	Length: 10.5 mm Outer radius: 1.6 mm Inner radius: 1 mm	Isotropic 1e-10 S/m
Sieve polyimide	Outer radius: 1 mm	Isotropic 1e-12 S/m
Sphere (model of surrounding tissue)	Radius: 5.5 cm	Isotropic 2 S/m

The initial potential of all objects in the model was set to zero. The model was computed twice. In the first run of the model one electrode terminal in the proximal sieve was set to provide 1A of current over the surface area of that electrode terminal (1/3.263E-8 A/m^2) whereas the ground was set as the outer surface of the sphere encompassing the double macro-sieve assembly and the entire nerve.

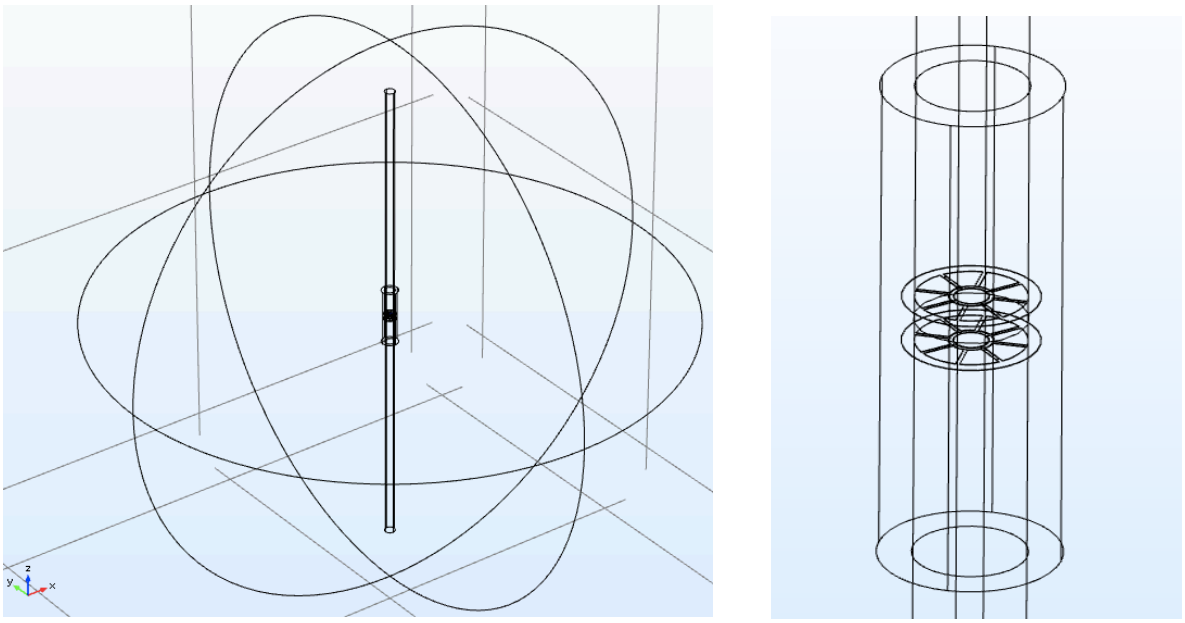


Figure 4.2: Comsol 3D model of dual macro-sieve assembly

In the second run, the current source of the electrode terminal was disabled and a second current source of 1A across the surface area of all 8 electrode terminals (1/3.263E-8 A/m^2) of the distal sieve was enabled. The ground was the same as in case of the stim – the surface of the surrounding sphere.

Each time the model was computed, the potential profile resulting from the current flow was extracted using Matlab (Figure 4.3)

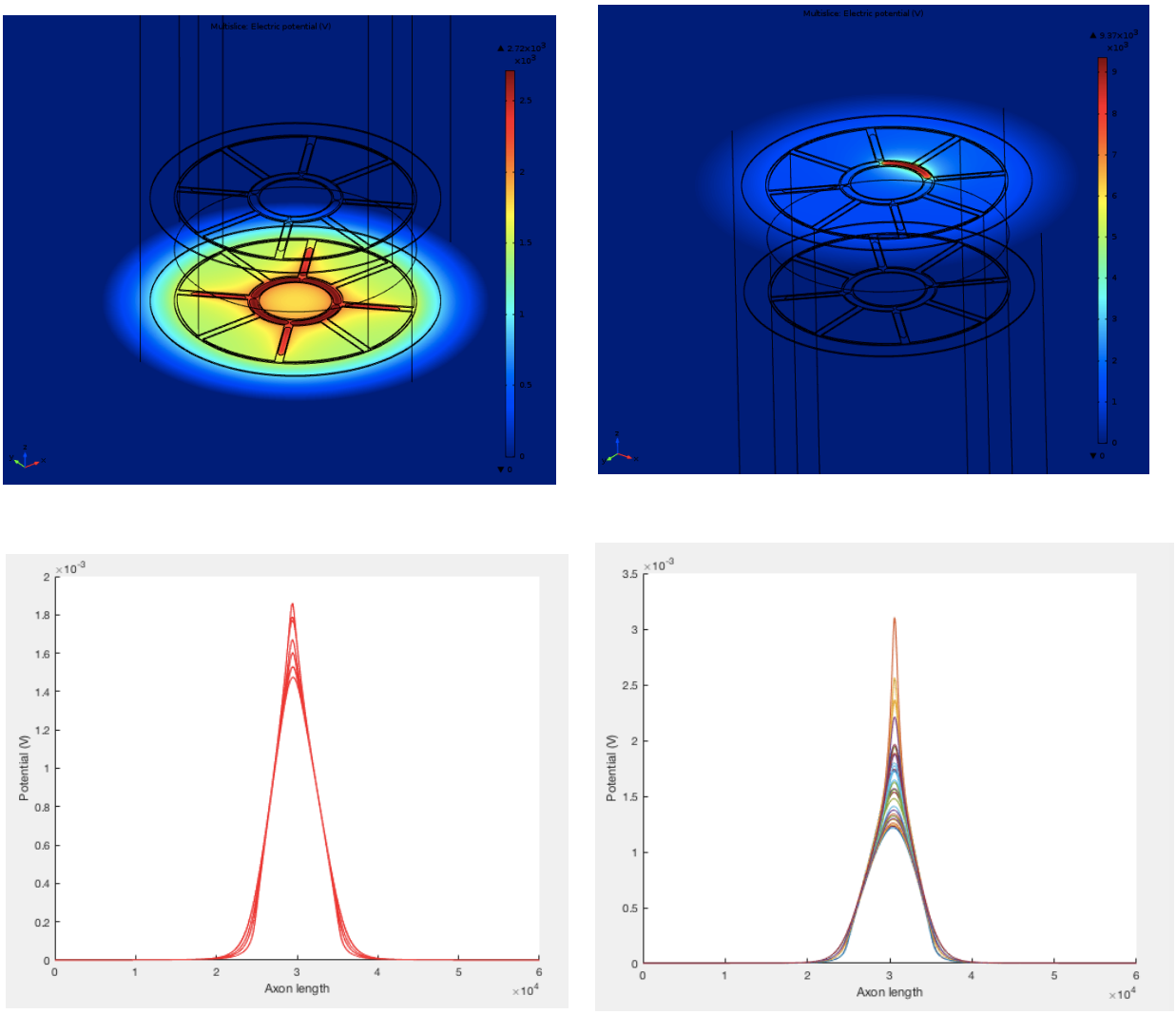


Figure 4.3: Potential profiles from Comsol. Top left – All eight channels of the proximal macro-sieve electrode is given the blocking signal and the model is computed. Top right – One channel of the proximal macro-sieve electrode is given the stimulus and the model has been computed. Bottom left – potential profile of 57 axons derived from the blocking signal. Bottom right – potential profile of 57 axons derived from the stimulus

Axon modelling was done in the Neuron software application. The CRSSS model was implemented with the following parameters

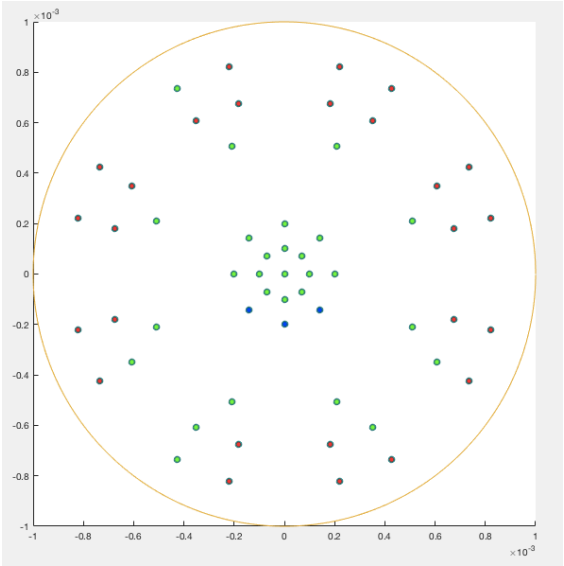
- Number of nodes: 101
- Number of axons sampled at distinct points in the cross section of the nerve: 57 (Figure 4.4 – top panel)
- Number of voltage profile points: 60000
- Stimulus duration: 0.2 ms
- Block current: 30uA
- Block frequency: 30kHz

4.2 Results and discussion

Of the 57 axons sampled across the cross section of the nerve,

- unidirectional block was achieved in 28 axons
- 3 axons were blocked bi-directionally
- no block was achieved in 26 axons

As has been observed experimentally, in experimental setup 1, where the references are placed far off for the stim and HFAC blocking signal, the block is not observed. In the simulation only a fraction of the axons across the cross section of the nerve are blocked. Both normal axons and regenerated axons (with their characteristic distribution of axon diameters – regenerated axons had a much higher proportion of low diameter axons) were used to run the simulation. In both cases the HFAC signal was not able to block the action potential in all the axons.



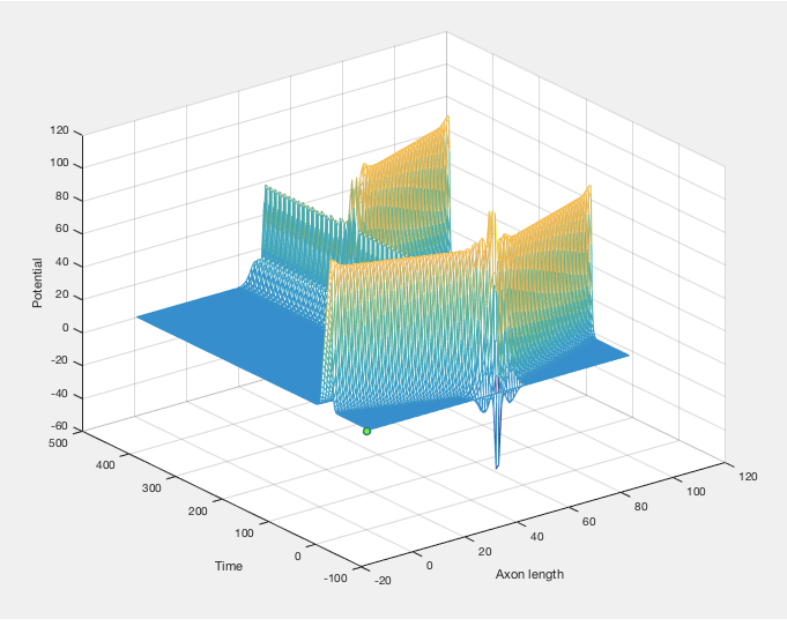


Figure 4.4: Results of axon modelling I. Top panel – the diagram shows the relative position of the 57 axons across the cross section of the nerve. Green indicated a complete unidirectional block. Red indicates no block was achieved. Blue indicates the signal was blocked bidirectionally. Bottom panel – example of an unidirectionally blocked action potential in an axon. The stimulus is provided 5 seconds after the HFAC block

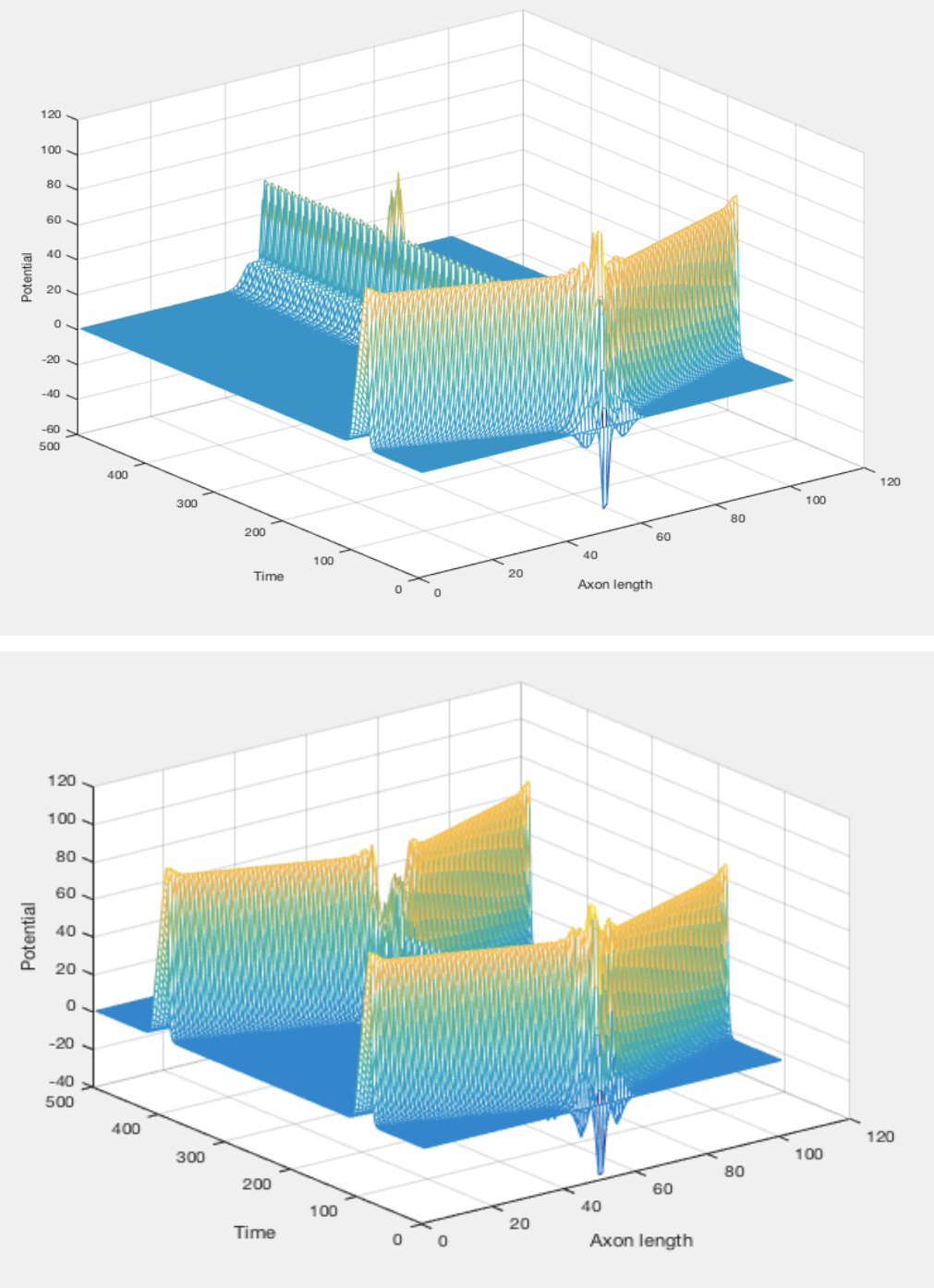


Figure 4.5: Results of axon modelling II. Top panel – bidirectionally blocked stimulus. Bottom panel – no block was achieved in this axon.

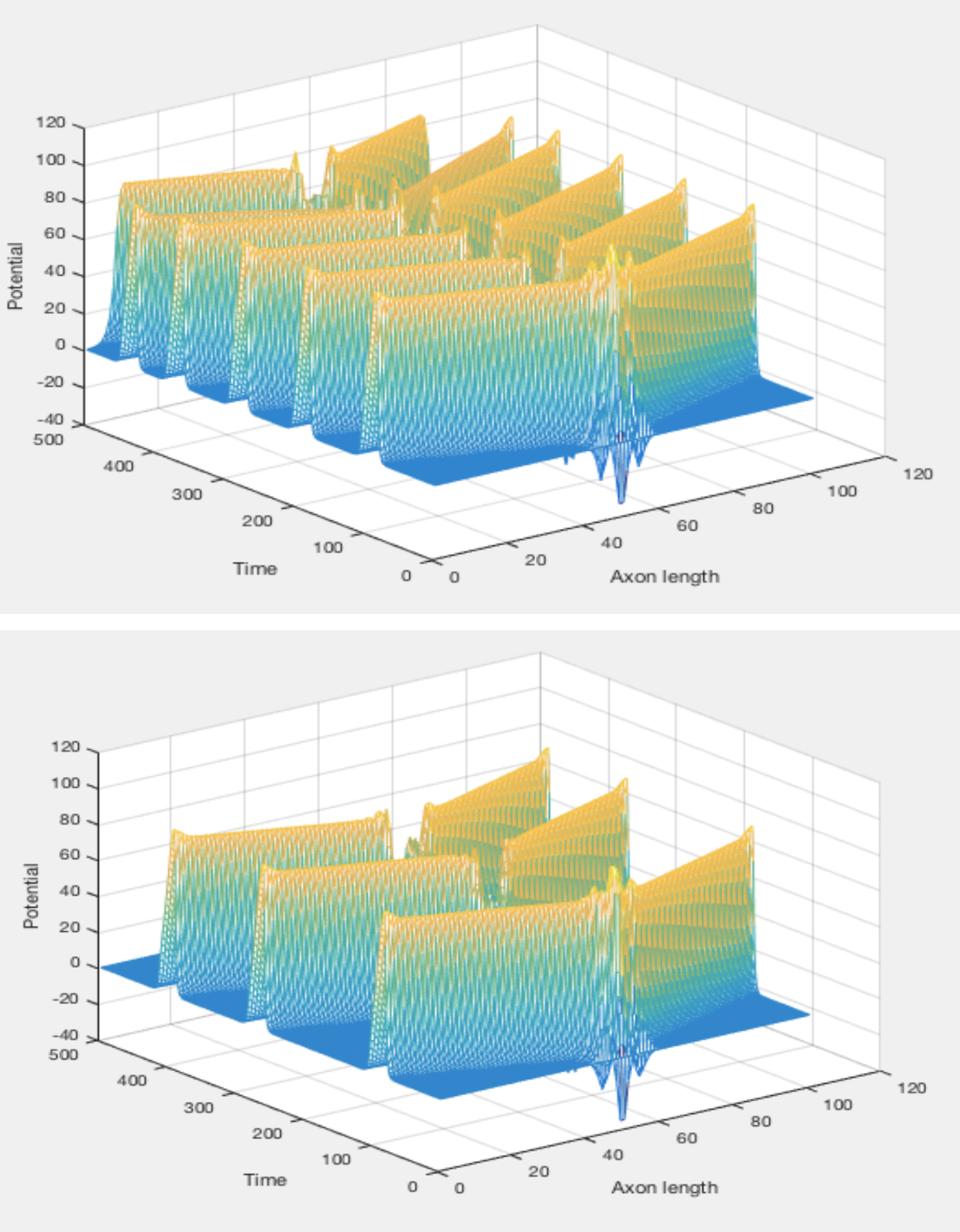


Figure 4.6: Results of axon modelling III. Top panel – excessive firing of the nerve post initiation of the block. Bottom panel – asynchronous firing post initiation of the block

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