

Preliminary Study of Time to Recovery of Rat Sciatic Nerve from High Frequency Alternating Current Nerve Block

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Abstract—High-Frequency alternating current nerve block has great potential for neuromodulation-based therapies. However, no precise measurements have been made of the time needed for nerves to recover from block once the signal has been turned off. This study aims to characterise time to recovery of the rat sciatic nerve after 30 seconds of block at varying amplitudes and frequencies. Experiments were carried out *in-vivo* to quantify recovery times and recovery completeness within 0.7s from the end of block. The sciatic nerve was blocked with an alternating square wave signal of amplitude and frequency ranging from 2 to 9 mA and 10 to 50 kHz respectively. To determine the recovery dynamics the nerve was stimulated at 100 Hz after cessation of the blocking stimulus. Electromyogram signals were measured from the *gastrocnemius medialis* and *tibialis anterior* muscles during trials as indicators of nerve function. This allowed for nerve recovery to be measured with a resolution of 10 ms. This resolution is much greater than previous measurements of nerve recovery in the literature. Times for the nerve to recover to a steady state of activity ranged from 20 to 430 milliseconds and final relative recovery activity at 0.7 seconds spanned 0.2 to 1 approximately. Higher blocking signal amplitudes increased recovery time and decreased recovery completeness. These results suggest that blocking signal properties affect nerve recovery dynamics, which could help improve neuromodulation therapies and allow more precise comparison of results across studies using different blocking signal parameters.

I. INTRODUCTION

Recent developments in neurotechnology have established new modalities for interacting with the body's nervous system. Electroceuticals have been promoted as a new type of therapy delivery with notable ventures such as Galvani Bioelectronics [1] and the Feinstein Institute [2], as well as other governmental programmes run by DARPA, e.g. SPARC and ElectRx [3]. The vision is to create a new generation of medical implants interfacing with the peripheral nervous system to deliver therapeutic stimulation. In many cases it is not sufficient to simply stimulate nervous activity but also to reduce or block it. The goal of stimulation is then not only to

excite nerve cells but also to inhibit them, paving the way for novel dynamic neuromodulation therapies. High Frequency Alternating Current (HFAC) nerve block is a promising technique for the latter, and is under active research [4]–[8].

Characterizing nerve recovery from HFAC block can lead to improvements in stimulation selectivity or stimulator power consumption, for example by exploiting recovery dynamics to reduce the duty cycle of blocking [9]. So far attention has been focused on measuring recovery dynamics from block when it is applied over long periods of time; 15 minutes or more [7], however investigation of 'fast' block recovery has been the subject of few studies. When block has been applied for less than 10 minutes, recovery times have been found to be less than 1 second [10]. However, more precise measurements of block recovery times with smaller resolutions have not been made. As continuous duty-cycling involves application of block for short durations, it becomes particularly important to precisely measure recovery dynamics in those conditions.

II. METHODS

A. Experimental Setup

All animal care and use procedures were performed under licences issued by the UK Home office under the Animals (Scientific Procedures) Act (1986) and were approved by the Animal Welfare and Ethical Review Board of Newcastle University.

Four Sprague-Dawley rats weighing 350–450 g were used. General surgery procedures followed those described in [11].

B. Instrumentation

EMG signals from *gastrocnemius medialis* and *tibialis anterior* were captured using tungsten wires connected to two channels of a differential bio-amplifier (Model 1700, A-M Systems, WA, USA) as shown in Fig. 1a. Stimulation was delivered through a custom-made nerve cuff (Microprobes for Life Science, Maryland, USA). The EMG signals were filtered using a 10 Hz high-pass second-order filter, a 10 kHz low-pass second-order filter. A 50 Hz notch filter was used to remove line noise. The amplifier gain was set to 100. The output of the differential amplifier was connected to two analog channels of a Cerebus Neural Signal Processor (Blackrock Microsystems, Utah, USA).

Two different devices were used to deliver stimulation. The sets of three contacts on each of the cuff's extremities were connected to a Cerestim stimulator (Blackrock Microsystems, Utah, USA) (monopolar), whereas the middle 8

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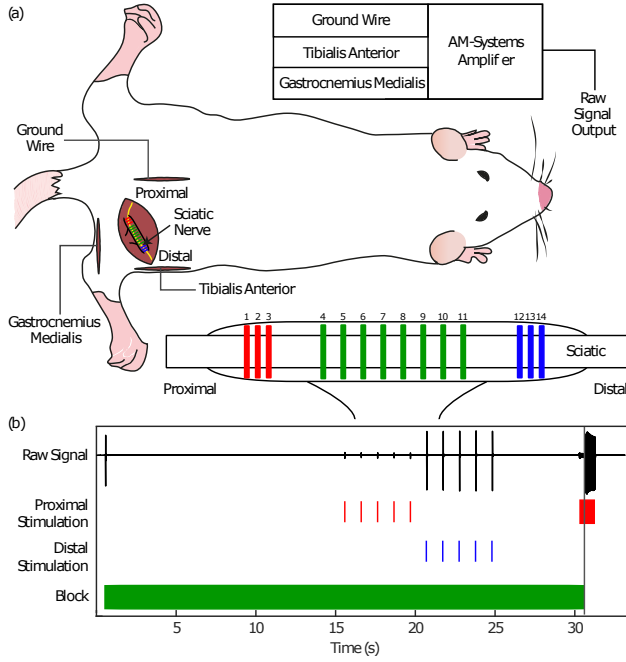


Fig. 1. (a) Experimental setup illustrating a Sprague Dawley rat and connections from nerve cuff to instrumentation. Connections to stimulating instruments not shown. The CereStim system delivered excitatory stimulation on cuff contacts 1-3 and 12-14. A custom PCB device delivered HFAC block on cuff contacts 4-11. The CereStim shared the ground reference with the A-M Systems amplifier. (b) Typical stimulation and block timeline showing raw EMG trace, proximal, distal and blocking stimuli, with blocking effect visible as evoked response is significantly reduced as long as block is active.

contacts were connected to the custom Printed Circuit Board (PCB) used to deliver HFAC block (bipolar) using current control. The CereStim system carried out stimulation distal and proximal to the area blocked by HFAC. EMG capture was synchronized with stimulation by use of a *sync* signal from the CereStim.

The schematic for the analog portion of the circuit is shown Fig. 2, with component values detailed in Table I.

Discrete blocking capacitors were used to prevent DC contamination of the blocking signal delivered by the custom PCB device. Switching and TTL signals were driven by a KL25z Microcontroller (Freescale) to ensure consistent timing during trials, and a MATLABTM(version: 16a) graphical user interface was used to communicate with the microcontroller. The gain and offset of current branches was calibrated by adjustment of the trim potentiometers, so as to deliver the same current with opposite polarity with the KL25z's onboard 12-bit DAC as common input. The custom PCB was powered by galvanically-isolated power supplies to ensure isolation between stimulators.

C. Protocol

Blocking signal frequencies spanned 10kHz to 50kHz in 5kHz steps, and amplitudes spanned 2mA to 9mA in steps of 1mA, except for rat 1 for which the highest tested amplitude was 8mA. In each trial, block was delivered for 30 seconds. Onset of block was confirmed by the recording of an

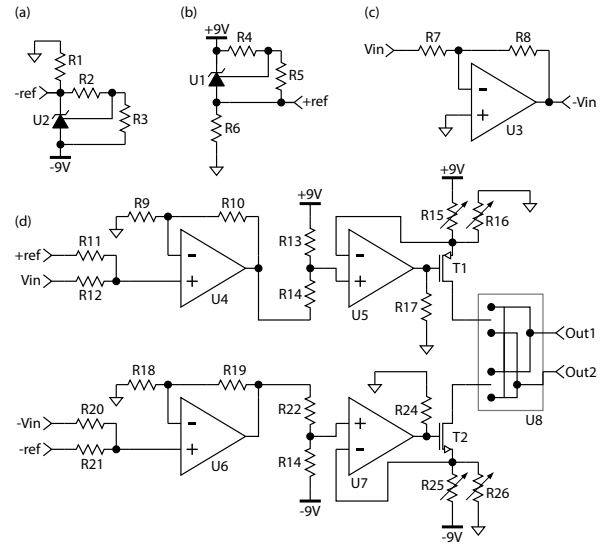


Fig. 2. Circuit schematic for the analog portion of the custom-made PCB device used to deliver the blocking signal. (a) and (b) are voltage references built using precision shunts; (c) is an inverter used to invert the input signal generated by the DAC for input into the sink branch of the stimulator. (d) is the core of the stimulator showing separate current source and sink individually calibrated with trim potentiometers. Connections between parts of the circuits are named using node references.

TABLE I

COMPONENT VALUES AND DESIGNATORS FOR FIG. 2. FOR RESISTORS (PARTS WITH 'R' DESIGNATOR), THE Ω SIGN IS DROPPED FOR BREVITY.

Part	Value	Part	Value	Part	Value
R1	5k	R10	10k	R19	10k
R2	10k	R11	10k	R20	10k
R3	14.7k	R12	10k	R21	10k
R4	10k	R13	1k	R22	27k
R5	15.4k	R14	27k	R23	1k
R6	5k	R15	50	R24	50k
R7	50k	R16	20k	R25	50
R8	50k	R17	50k	R26	20k
R9	10k	R18	10k	T1	BSS8402(a)
T2	BSS8402(b)	U1	LM4041	U2	LM4041
U3	OPA192	U4	OPA4192(a)	U5	OPA4192(b)
U6	OPA4192(c)	U7	OPA4192(d)	U8	ADG1433

onset response. During block, five proximal pulses followed by 5 distal pulses, delivered at 1 Hz were used to evoke EMG. Proximal pulses were used to control for block quality, and distal pulses were used to control for muscle fatigue. Subdued or absent EMG response proximally and strong, consistent response distally indicated good quality block. Stimulation was started 15 seconds after the onset of block to ensure the onset response had subsided. Approximately 0.3 seconds before block was stopped, the nerve was stimulated proximally at a rate of 100 Hz in order to capture nerve recovery with 10 ms precision. Signals were verified to be EMG by the delay between the stimulation and the signal, with the presence of minor stimulation artefacts due to the large distance between the stimulating cuff and the tungsten wires in the muscle. In order to minimise the effect of

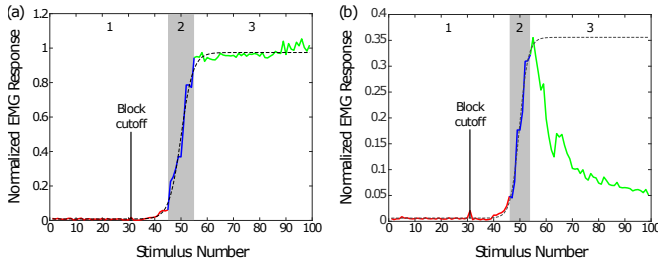


Fig. 3. (a) Sigmoid fit curve for the recovery of *tibialis anterior* from block at 20 kHz and 7 mA. (b) Sigmoid fit curve for the recovery of *gastrocnemius medialis* at 25 kHz and 7 mA. The three recovery regions are marked using numbers and grayscale overlay. Note the different scales in both plots, corresponding to different degrees of recovery for each muscle for different frequencies.

fatigue, enough time was allowed to elapse between trials to ensure that each muscle had recovered, and baseline muscle response was measured regularly. A timeline with an example recording is shown in Fig. 1, for a trial with block at 15 kHz and 6 mA. To prevent biasing of the results by a drifting baseline over the duration of the experiment, the compound muscle action potential (CMAP) of the muscle was measured at regular intervals in trials with no block, to correct for any potential deviations. Values for muscle activity during block recovery were normalized against these measures.

D. Data Analysis

To quantify the dynamics of recovery from block, the area under the curve of the normalised, rectified EMG response is measured for each spike in the 100 Hz stimulation train and is then fitted to a sigmoid curve with three regions corresponding to the lowest 10% of values, the middle 80% and the highest 10%, with examples for *tibialis anterior* and *gastrocnemius medialis* shown in Fig. 3. Only the trials for which the fitting curve has an adjusted R-square value of more than 0.9 were plotted in the final results. This ensured that the data is not biased by incomplete block, which often leads to muscle fatigue. The recovery time was measured as the time between block cut off, and when the normalized EMG response reached a steady state or plateau. As *gastrocnemius medialis* was particularly prone to fatigue during stimulation in the recovery phase, the maximum value of the measured recovery curve was used to fit for the maximum value of the sigmoid curve to reduce biasing of the result as shown Fig. 4.

III. RESULTS

When considering averaged values in the data, shown Fig. 4, the results show that block recovery time and recovery completeness within 0.7s of cessation of block can be affected by blocking stimulus parameters. Increasing the blocking signal amplitude leads to a stronger blocking effect, and increases the time needed by the muscle to recover, regardless of frequency (Fig. 4, a). Note that when the recovery delay is approximately 0.7 seconds this indicates the muscle did not recover completely within the time

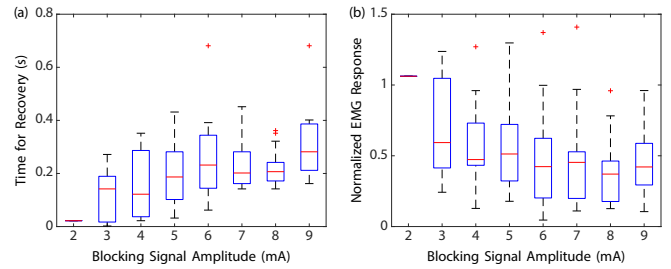


Fig. 4. (a) Boxplots of block recovery delay in seconds versus blocking signal amplitude, collated across frequencies and both *gastrocnemius medialis* and *tibialis anterior*; (b) Boxplots of final normalised EMG response at the end of the 100 Hz stimulation train versus blocking signal amplitude, collated across frequencies and both *gastrocnemius medialis* and *tibialis anterior*.

scale of the trial. Similarly, very low (under 0.25) final normalised EMG responses (Fig. 4) indicate low extent of recovery during the corresponding trial. For trials in which the adjusted R-square error for the sigmoid fit for muscle recovery was greater than 0.9, recovery times ranged from 20 to 430 ms, with most trials within the 100 to 300 millisecond range. Final normalized EMG response measures displayed more variance, due to different extent of recovery between rats 1 and 2, with lower recovery, and rat 3, with higher recovery overall. Increasing block signal amplitudes led to lower final normalised EMG response for rats 1 and 2. For rat 3 however, final normalised EMG response was insensitive to block amplitude and decreased with increasing block signal frequency (not shown in collated results).

IV. DISCUSSION

Block was delivered to the sciatic nerves of rats and the recovery time measured by fitting relative EMG values from stimulation to a baseline measurement. Three regions for recovery were identified: an initial region where the muscle does not recover, a region where the muscle recovers quickly to a peak relative EMG value, and a final steady state region where the muscle does not achieve higher recovery. Individual measurements were highly variable from animal to animal, nevertheless time to recovery from short-duration block was measured with greater precision than before, spanning 20 to 430 ms. Measurements varied more for *gastrocnemius medialis* than *tibialis anterior* across all three rats. This may be due to differences in the fiber composition of nerves innervating the two muscles, or specificities of the *in-vivo* preparation. In addition, the sparse data set had to be collated for both muscles and across frequencies. This is a result of filtering for trials (see subsection II-D) in which good quality block was observed.

Furthermore, some variance may be attributed to incremental changes in the surgical protocol during the experiments in an attempt to improve the contact quality between cuff and nerve. Compared to the first two rats, measures for rat 3 were more consistent, suggesting that further refinement of the protocol could yield clearer trends in the results. Notably, for rat 3 the cuff was closed around the nerve using separate loops of suturing thread rather than the sutures

embedded in the cuff, which resulted in a tighter fit and the ability to recover the cuff without cutting its own sutures. The resulting assembly inside the surgical cavity was stabilised with Kwik-Cast (World Precision Instruments).

As mentioned previously, the fitting algorithm applied to the recovery curve of *gastrocnemius medialis* was made to use the highest value observed as the final recovery value, which increased fit quality to acceptable levels while minimising bias from early onset fatigue. While this effect could have been avoided with lower stimulation frequency during recovery, this would have worsened time resolution for the measurement of recovery delay, as it was not possible to spread the measures over more trials due to experimental time constraints. In trials for which the muscles did not recover completely within the 0.7s time frame, subsequent controls showed no difference in baseline excitability for trials without block. Lengthening the recovery phase to capture particularly slow recovery would have accentuated the effects of fatigue and was not attempted.

Finally, in some trials cessation of block apparently led to a sharp decrease in the muscle's EMG response. This is contrary to expectations, which are that the muscle starts to recover as soon as block is turned off. A subsequent literature search after the experiments revealed that charge accumulation on the blocking capacitors used with the custom PCB device could lead to DC pulses being delivered at the start and end of block, which could explain these observations [12]. In this case block recovery times and final normalized EMG response may be lengthened and reduced respectively.

Nevertheless, trends are evident when considering the average values over collated data. Increasing the block amplitude increases the charge delivered per cycle of the blocking waveform, leading to dose-dependent increases in recovery time and reductions in final recovery value as observed by Bhadra [7]. No consistent effect was seen for changes in frequency as measurements from rat 3 differed from those of rat 1 and 2. DC pulsing effects may have affected the results for the lowest frequencies, and it is expected that DC pulsing is stronger with increasing block amplitude and decreasing frequency. Further work could attempt to more effectively separate the effects of the two variables by normalising results with respect to injected charge per cycle of the blocking signal.

Depending on fibre composition and activation function differences between sciatic trunk and GM or TA-specific branches it is possible that EMG waveforms would have been different if the respective nerves innervating the TA and GM muscles had been cuffed separately. This was not attempted due to the surgical complexity of the experimental setup. For the same reason only one nerve per rat was able to be used within the time frame of the experiment.

V. CONCLUSION

In this study the recovery from HFAC block of the *gastrocnemius medialis* and *tibialis anterior* muscles of the rat was measured with a resolution of 10 ms, capturing the

recovery process with greater precision than previously. The curve obtained from measuring the integral of the absolute value of EMG responses to stimuli during and after block was fitted to a sigmoid which enabled the identification of 3 regions of recovery. The initial region is of low EMG activity and corresponds to the portion of the stimulus train delivered during block. The second region captures the recovery of the muscle from block, and the third corresponds to the steady state of muscle activation after the recovery period.

Results for both muscles were collated to provide trends using box plots observed in the data, showing that as the blocking signal amplitude is increased, the recovery time increases and the normalised EMG response after recovery decreases. This indicates that the nerve recovers more slowly as blocking signal amplitude is increased. The variance displayed in the results motivates further work to refine the protocol and validate observed trends.

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