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The evoked compound nerve action potential is shaped by the electrical pulse-width

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Abstract: Introduction: Despite its central role in medicine electrical stimulation (ES) is still limited by its selectivity. Different reports did assess effects of different waveforms, intensities, and frequency on the activation threshold of nerve fibres with different diameters. We aimed to extend this knowledge by investigating the effect of short monophasic rectangular pulses (1, 2, 5, 10, 50, 100 and 200 μs) on the recruitment order. **Methods:** The sciatic nerve of rats was stimulated, and the evoked compound nerve action potential (CNAP) measured at two sites on the tibialis nerve, using epineural electrodes. Changes in delay, amplitude, and the shape of the CNAP were analyzed. **Results:** The amplitude and delay of the CNAP were significantly affected by the pulse-width (PW). The delay and duration of the compound nerve action potential increased with longer PW, while the amplitude decreased. **Discussion:** Found changes are likely caused by changes in the time point of excitation of individual neuron fibres, depending on electrical field strength and exposure time. This might be of particular interest when selecting PWs for design and validation of stimulation patterns and analysis of experimental and clinical observations.

Keywords: electric stimulation (ES), compound nerve action potential (CNAP), pulse-width (PW), epineural electrodes

Introduction

One of the fundamental principles of ES is the observation that the excitation threshold of a nerve fibre is inversely proportional to the PW. This was already described by Weiss and Lapicque more than 100 years ago [1], and yet, the significance of the PW in practical applications is often underestimated. Grill and Mortimer [2] tested rectangular PW of 500, 100, 50, and 10 μs in computer simulations and validated the results with in-vitro and in-vivo measurements. They concluded that a PW below 100μs allows for more spatially selective activation of neurons and increases the threshold differences between fibre types. Gorman and Mortimer [3] concluded that PW below 10μs would increase the threshold differences even more, but suggested 10μs as a reasonable limit for neural stimulation [3]. On the other hand, longer PWs produce stronger muscle contractions and deeper penetration below the skin [4].

This study aims to provide more in-depth knowledge of the effect of PW under 10μs and to compare them with more typical durations in terms of their effects on the recruitment of nerve fibres. With this purpose, an exploratory proof of principle study in an in-vivo model was performed at the Department for *Sport and Exercise Sciences* of the *John Moores University* in Liverpool.

Methods

All experiments were carried out under strict adherence to the Animals (Scientific Procedures) Act of 1986. The procedures were approved by the Home Office (PPL 40/3743) and were conducted in four non-recovery experiments in adult Wistar rats.

Anaesthesia was induced using 3% isoflurane in oxygen. To maintain stable, deep anaesthesia, the respiration rate was monitored, and the isoflurane concentration was adjusted between 1% and 2%. The body temperature was kept between 37-38°C with an adjustable heat pad (E-Z Systems Corporation, Pennsylvania, USA), and the core temperature was monitored using a rectal temperature probe. 0.05 mg kg−1 of Buprenorphine (Temgesic, Indivior, Slough, UK) was administered intramuscularly in the contralateral leg for analgesia. For stimulation, a 1.2mm diameter tripolar cuff electrode (Micro Cuffe Tunnel, Cortec, Germany) was placed proximal to the tibialis branch on the sciatic nerve. For monitoring, two 0.6mm diameter bipolar cuff electrodes (Micro Cuffe Tunnel, Cortec, Germany) were placed on the tibialis nerve with a 1mm distance between them (see Figure 1).

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Stimulation pulses were generated at a resolution of 1MS/s using LabVIEW 2016 (National Instruments Corporation, Austin, USA). Voltage-controlled stimulation was applied using the analogue output of a NI PCIe 6351 Data Acquisition Card (National Instruments Corporation, Austin, Texas, USA) with ±10V output range, 5mA output current drive, 20V/µs slew rate and 16bit DAC resolution. The stimulation artefact was minimised by applying the stimulation via tripolar cuff electrodes using the middle electrode as cathode and interconnected proximal and distal electrode surfaces as anodes.

Stimulation sets with PWs of 100, 50, 20, 10, 5, 2, and 1µs were tested. All pulses were monophasic. After an initial trial with 50 repetitions per duration, the subsequent assessments were done with 100 repetitions to increase the resolution. Amplitudes ranged from sub-threshold to full nerve activation. The threshold and supramaximal intensity for each set and subject were determined by single test stimulations. At least 40 or 85 different intensities were then tested within the selected range. The different stimulation PW and amplitudes were applied in a randomised order. The data were normalised by applying a supramaximal pulse – monophasic with 100µs PW – every 20th stimulation. The electroneurogram (ENG) signals were recorded using a PowerLab 16/35 (ADINSTRUMENTS Ltd, New Zealand) acquisition unit controlled with LabChart (ADINSTRUMENTS Ltd, New Zealand) at a sampling frequency of 200kHz.

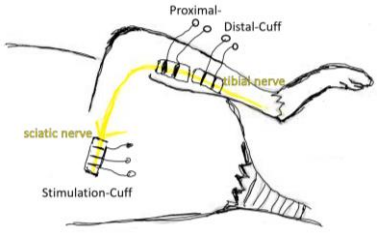

Figure 1: Measurement setup with the tripolar stimulation cuff at the sciatic nerve and the two bipolar measurement cuffs at the tibial nerve.

The ENG was differentially amplified, using the proximal electrode as anode and the distal electrode of the same cuff as cathode for both recording cuffs. Shielded copper wire cables with two conductors were used to connect the cuff electrodes to the before mentioned amplifier for the ENG recording. All shields were connected to a metallic rectal probe which served as reference ground for the differential amplifier.

Data processing, analyses, and visualisation were done using MATLAB R2019b (The Mathworks, Inc., US).

Three points of interest (POI), P1, N1, and P2, according to the expected waveform of an evoked ENG response described by Parker [5], were determined in the recorded traces (see Figure 2a).

The response amplitude was defined as the difference between P1 and N1, similar definitions can be found in [5], and it was normalised relative to the nearest control response. The normalisation responses were checked over time to see if there was a time-dependent effect of the stimulation or the prolonged anaesthesia.

The delay between the stimulation onset and P1 was calculated. For each subject and PW the difference between the appearance of P1 in the proximal and distal channel was calculated. Three-way ANOVAs were conducted to evaluate the effect of stimulation amplitude, PW, and subject on the delay between stimulation and P1, the time difference between the appearance of P1 in the proximal and distal channels (proportional to the conduction velocity), as well as on the duration and amplitude of the CNAP. The duration of the CNAP was defined as the time difference between P1 and P2.

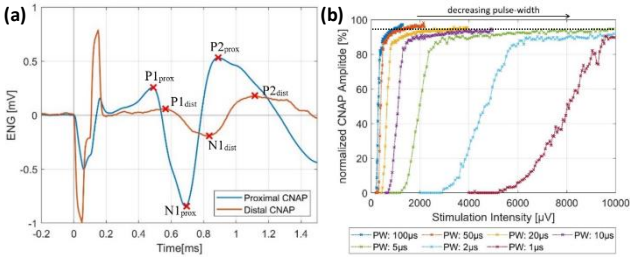

Figure 2: (a) Example of a measured response with the marked points of interest (P1, N1, and P2) in the proximal and distal cuff electrode (stimulation parameters: PW=100µs; Amplitude=1.289mV) **(b)** Normalized recruitment curve of the proximal cuff of the CNAP responses of a single subject for different PW. The black dotted line visualizes that for this subject PW 20µs and 5µs saturate at nearly at the same level

Results

The delay of the neural responses was between 280µs and 535µs ($\bar{x}$=414±38µs n=2114), after the stimulation and lasted between 140µs and 570µs ($\bar{x}$=369±74µs n=2114) depending on the subject, PW, and stimulation amplitude. Both the mean duration of the CNAP and the mean delay of the response decreased with decreasing PW. The latencies of P1, N1 and P2 were always longer in the distal channel (see example in Figure 2a).

Delays between stimulation onset and P1 got shorter with decreasing PW. However, this effect becomes undetectable for PW smaller or equal to 10µs. Furthermore, this effect decreases with higher recruitment (supra-threshold intensity).

The calculated conduction speed varied between ~35 and ~87m/s. The intersubject variability accounted for most of the difference in the speed. This might be due to physiological differences and differences in the distance between the electrodes. Due to poor detection of P1 in the distal cuff within one subject, the data of this subject was not used to calculated conduction speed. The influence of the PW and of the stimulation amplitde were not significant. On the other hand, the delay of P1 was significantly influenced by the PW and the subject, but

not by the stimulation amplitude (see Table 1). The normalised CNAP amplitude and its duration was significantly affected by all three factors (see Table 1).

Table 1: Three-way ANOVAs conducted on the effect of PW, amplitude and subject on P1 conduction velocity and latency, and the CNAP amplitude. DF=degrees of freedom. * DF=2

	PW DF= 6	**stim. amplitude DF=1**	**Subject DF=3**
Conduction P1 Error=1378	F=0.23 p=0.97	F=1.48 p=0.22	F=6839.39 p<<0.01*
delay P1 Error= 2096	F=379.97 p<<0.01	F=0.15 p=0.70	F=4614.11 p<<0.01
CNAP amplitude Error= 2103	F=276.81 p<<0.01	F=1188.52 p<<0.01	F=25.83 p<<0.01

Longer PWs tend to cause a longer delay of P1. With higher recruitment, the duration of the CNAP response increased for all subjects.

Figure 2b shows the normalised recruitment curve (RC) for all different stimulation PW in one subject. As results were similar in both distal and proximal positions, only the normalised CNAP amplitudes from the proximal cuff are reported. In both cases, the response amplitude of the control pulses did not change significantly with time, showing that there was no time-dependent bias of the results.

Stimulation amplitude ranges for each PW to reach certain response levels are remarkably similar in all subjects. The saturation of longer PW is steeper compared with shorter PW. This can be seen in a high asymmetry of the recruitment curve, especially for longer PW, caused by a more linear rise, after the recruitment starts to saturate. Hence, PW do seem to finally saturate at nearly the same level (see black dotted line in Figure 2b). However, none of the responses reaches 100%. This is due to supramaximal stimulation used as normalisation pulses. The stimulation step size increases with shorter PW, as the difference between below threshold to "full activation" increases with decreasing PW.

Discussion

Estimation of recruitment of neurons of different type, size and conduction velocity in a mixed nerve by applying specific electrical stimuli is a complex task and, despite the long history of electrical stimulation, not resolved to a sufficient extent. Here we present an in-vivo model with cuff electrodes placed along a rat's sciatic nerve for stimulation and ENG-recording, to explore the influence of unusually short PW (1 to 100µs) on neuron recruitment characteristic by amplitude variation, in 4 animal subjects.

All recorded ENGs in the 4 subjects in the proximal as well as the distal channel had a similar shape, consistent with published results on CNAP shape and the descriptive parameters amplitude, latency, and duration [5-7]. Assessed conduction velocities were within the expected range for sciatic nerves of rats [8]. Differences in delay of evoked CNAPs, attributed to variations in anatomical distances were verified and considered in calculations and interpretations.

Since neither the PW nor the stimulus amplitude affected the conduction velocity, we assume that differences in the delay of P1 are mainly due to changes in the time point of excitation between different PW. Hence, the duration of the CNAP, calculated via the time difference of P1 and P2 in the proximal channel, and the time difference between P1 and N1, are comparable across subjects.

The shape of a CNAP is influenced by several factors, as it is a projection of multiple single fibre action potentials (APs) with different conduction speed and sagittal distance to the recording electrode. The longer the distance from starting point to recording site the broader the variance of arrival time of contributing APs, with immediate consequences for P1-latency, -amplitude, and the CNAP duration. Another influence on CNAP shape is contributed by the range of distances from individual fibres to the recording electrode. An additional factor is variation in the time from the stimulus leading edge to the start of a propagating AP, which depends on fibre size and local field strength (see Figure 3b).

The saturation level in Figure 2b shows a gradual increase of maximum CNAP amplitude with increasing PWs. This might originate from exciting less small unmyelinated and distant fibres with lower PW due to extremely increased amplitude threshold.

Figure 2b also illustrates a significant effect of PW on fibre threshold and gradient of the RC slope; shortening of PW strongly increases threshold amplitude. Although a similar relation can be found in classical strength duration curves, obtained via sensory perception feedback or neuromuscular reactions [9], the results from direct neural response recording suggest that more pronounced changes in selectivity neuron types occurs with lower PWs.

For a certain pulse amplitude, an "excitation window" EW can be defined, showing the range of pulse durations for which, this amplitude will excite different quality fibres. It starts at the threshold of the most sensitive, nearest-to-electrode, large axons and extends to the threshold of the most distant, small size and/or non-myelinated axons. Two examples are labeled in red and blue (red with 2 different amplitude levels) in the threshold curves in Figure 3b.

Within an EW a stimulus of a certain length and amplitude activates action potentials in each reachable neuron. Taking

this into account, the observed differences in recruitment and CNAP shape, elicited by variation of PW, are most likely not only due to size-dependent fibre involvement, but also by a distance-to-electrode component. This is probably a specific feature of epineural electrode placements. Although not evaluated here, we expect that the distance-to-electrode influence gradually disappears with greater distance.

A further influence on CNAP is associated with asynchrony of arrival of contributing single fibre APs, elicited in synchrony but traveling with different velocities. This effect is also specific for epineural electrodes and gets more diffused with larger neuron-to electrode-distance and higher desynchronization of fibre APs.

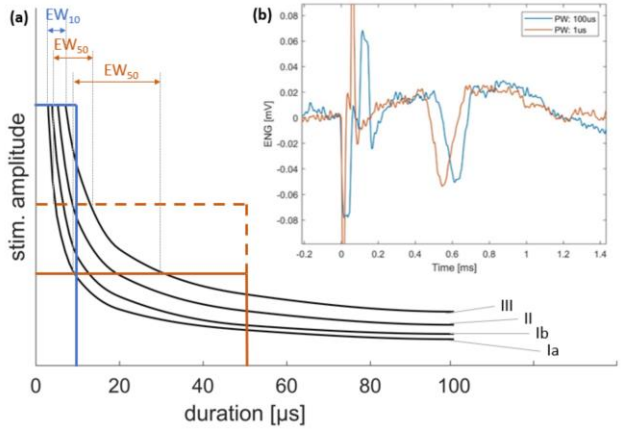

Figure 3: **(a)** 5: Illustration of strength-duration curves with thresholds for different types of nerve fibres (Ia, Ib, II & III). Two different stimulation PW (blue 10µs and orange 50µs) have different windows of excitation (EW). **(b)** Exemplary recording of responses for two different PW in the proximal cuff electrode.

Figure 3a represents strength-duration curves for different fibre types and helps to identify the excitation windows of PW different intensities (EW20, EW100). The activation window is defined as the time where the first fibre is triggered (e.g. fibre Ia) until the last fibre is activated (Fibre III), and it is implied in the strength-duration curve described by Lapique more than a century ago.

The presence of the excitation window can already be seen in strength-duration behaviour for different sized fibres (see Figure 3a). Higher amplitudes (blue rectangle) decrease the delay between stimulation stimulus onset and threshold (in this example Ia fibres) and decrease the length of the excitation window (e.g. time between reaching the threshold for fibres type Ia and III). Higher synchronisation leads to an additional increase of CNAP amplitude.

In conclusion, CNAP amplitude alone is not specific enough to reliably infer the recruited fibre pool when responses to different stimulus parameters are investigated. Other parameters in the CNAP shape can provide additional meaningful information for better estimation of recruited neurons in a specific setup and parameter set. Here we demonstrated that latency and duration of the response complement information based on changes in amplitude. The findings are most relevant for electrodes directly attached to the epineurium. Other more distant electrode configurations require specific studies for better understanding of anatomical and physiological interaction with artificially induced ES fields.

As suitable methods for selective in-vivo activation of single neurons and recording from single nerve fibres are not in sight for the foreseeable future, critical analysis of bio signal recordings in meticulously target-oriented experimental setups seem currently most promising approaches for gaining more detailed insight in mechanisms of ES of neural structures.

Author Statement

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