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# The Effect of Sub-Threshold Pre-Pulses on Neural Activation Depends on Electrode Configuration

# The Effect of Sub-Threshold Pre-Pulses on Neural Activation Depends on Electrode Configuration

Steffen Eickhoff and Jonathan C. Jarvis

***Abstract*— *Objective:*** **Published research on nerve stimulation with sub-threshold conditioning pre-pulses is contradictory. Like most early research on electrical stimulation (ES), the pioneer work on the use of pre-pulses was modelled and measured only for monopolar electrodes. However, many contemporary ES applications, including miniaturized neuromodulation implants, known as electroceuticals, operate in bipolar mode.** ***Methods:*** **We compared depolarizing (DPPs) and hyperpolarizing (HPPs) pre-pulses on neural excitability in rat nerve with monopolar and bipolar electrodes. The rat common peroneal nerve was stimulated with biphasic stimuli with and without ramp and square DPPs or HPPs of 1, 5 and 10ms duration and 10% - 20% of the amplitude of the following pulse.** ***Results:*** **The effects were opposite for the monopolar and bipolar configurations. With monopolar electrodes DPPs increased the amplitude required to activate 50% of the motoneuron pool (between 0.7% and 10.3%) and HPPs decreased the threshold (between 1.7% and 4.7%). With bipolar electrodes both pre-pulse types had the opposite effect: DPPs decreased thresholds (between 1.8% and 5.5%) whereas HPPs increased thresholds (between 0.5% and 4.1%). Electroneurograms from the stimulated nerve revealed spatial and temporal differences in action potential generation for monopolar and bipolar electrodes. In bipolar biphasic stimulation, excitation first occurred at the return electrode as a response to the transition between the cathodic and anodic phase.** ***Conclusion:*** **These data help to resolve the contradictions in the published data over two decades.** ***Significance:*** **They also show that fundamental research carried out in monopolar configuration is not directly applicable to contemporary bipolar ES applications.**

***Index Terms* — Action potential, Bipolar stimulation, Electrical stimulation, Sub-threshold pre-pulses**

## I. Introduction

Electrical stimulation (ES) is a neuromodulation technique that applies artificial electrical stimuli to alter the activity of target nervous structures. The stimuli are delivered via an active electrode near the target nerve. The charge can either be returned via a second electrode, often of similar size and situated in similar proximity to the target structure, this configuration is clinically called *bipolar*. If the charge is returned over a large, remotely placed electrode area, such as the casing of an implant, the configuration is known as *monopolar*. Although there are other electrode configurations, such as focused multipolar electrodes, monopolar and bipolar setups are the most commonly used in contemporary neuromodulation devices [1]. For some applications of ES either electrode setup might be used, but for others specific requirements predetermine the electrode configuration. For example, in the emerging field of miniaturized neuromodulation implants, so called "*electroceuticals*" [2] the electrodes are envisaged as integral with the stimulator. The small device size does not allow substantial electrode separation, so the configuration is inevitably bipolar. The same limitation applies to electrode arrays in which the maximum separation of electrodes is not much greater than the size of the target neural structure.

Beside the fundamental function of ES, to activate (or block) the target nerve, stimulation safety, selectivity and efficiency are key performance requirements [3]. The pursuit of stimulation selectivity, that is the activation of a specific neuronal population without coactivation of other fibers (for example, activation of sensory in preference to motor fibers, or slow motoneurons in preference to fast motoneurons), led to numerous investigations of ES with waveforms varying from standard rectangular pulses. One such modification is the addition of a sub-threshold conditioning pre-pulse immediately prior to the stimulating pulse. Sub-threshold means that the pre-pulse alone does not elicit action potentials (APs). Published data on the effect of such sub-threshold pre-pulses in computer simulations and various experimental settings are apparently contradictory [4]. Mortimer and Grill described in 1995 an effect of hyperpolarizing pre-pulses (HPPs) to decrease threshold [5] and an effect of depolarizing pre-pulses (DPPs) to increase threshold for activation [5]–[7]. Over the following two decades, several studies agreed with these original findings [8]–[10], but other research groups reported opposing results, describing a decrease of stimulation threshold with DPPs [4], [11]–[13].

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Like most early research on pulse shaping or fundamental mechanisms underlying electrical stimulation, the pioneer work by Mortimer and Grill was modelled and carried out only for the monopolar electrode configuration. To the best of our knowledge the transferability of findings from these investigations of the effect of DPPs and HPPs in monopolar electrode configurations to the bipolar case has not been studied. This represents a procedural gap in knowledge in the scientific literature on the effect of pre-pulses, especially in the light of the emerging field of miniaturized, and thus bipolar, neuromodulation devices.

The aim of this study was to investigate the effect of DPPs and HPPs on motor nerve recruitment. The pre-pulses were studied under conditions relevant to neuromodulating implants: The stimulation pulses preceded by DPPs and HPPs were biphasic and of a phase width near the chronaxie, that is, near the stimulus duration that uses the least energy to activate the nerve. Both principal electrode configurations, bipolar and monopolar, were tested and for the first time, detailed comparisons of the distal electroneurogram (ENG) of the stimulated nerve were made.

## II. Material and Methods

### A. Surgical Procedure

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 five 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 adjusted between 1% and 2%. 0.05 mg $kg^{-1}$ of Buprenorphine (Temgesic, Indivior, Slough, UK) was administered intra muscularly for analgesia. The body temperature was kept between 37-38°C with an adjustable heat pad (E-Z Systems Corporation, Palmer, Pennsylvania, USA).

Stimulation and ENG recording electrodes were made from PVC insulated stainless steel wire (Electrode wire AS634, Cooner Sales Company, Chatsworth, California, U.S.A.). Loops of 1mm diameter were formed from the uninsulated wire ends and placed in the tissue immediately underneath the common peroneal nerve (CPN) (Fig.1.a). Two stimulation electrodes were placed 2mm apart, approximately 5mm distal to the CPN branch from the sciatic nerve. The active electrode was the more distal electrode of the pair and the proximal electrode served as the return for bipolar stimulation. For monopolar stimulation the nerve return was not connected and a hypodermic needle (21G x 1-1/2") under the dorsal skin of the animal was used as a remote return electrode. A second electrode pair, also 2mm apart, was placed approximately 10mm distal to the stimulation electrodes and used for ENG recording, with the more distal electrode as reference (Fig. 1.a).

After freeing the extensor digitorum longus (EDL) muscle by dissecting the distal tendon of the overlying tibialis anterior, the proximal EDL tendon was clamped at the knee joint with an artery forceps which was firmly mounted to a steel table. The distal EDL tendon was dissected, fixed to a miniature titanium alloy hook, and connected to a force transducer (Gould Inc, Statham Instrument Division, Oxnard, California, U.S.A.). This procedure allowed us to mechanically isolate the EDL muscle and record isometric contractions, while blood supply and innervation were preserved. The proportion of isometric force generated was taken as indicative of the proportion of neural activation among the population of motoneurons within the common peroneal nerve that supply the EDL muscle (Fig. 2.b). The muscle was set to optimal length by increasing the length from slack in 0.5mm increments to the point where single stimuli elicited a maximal force response without excessive passive muscle tension [14]. Heated paraffin oil was delivered by a peristaltic pump (Watson-Marlow Ltd., Falmouth, Cornwall, UK) to the EDL surface at a rate of 0.1ml $min^{-1}$ to maintain a physiological temperature of 37-38°C and prevent the muscle from drying. Sterilized saline solution (OXOID Ltd., Basingstoke, Hampshire, UK) was administered subcutaneously to replace normal fluid loss during the surgical procedures (approximately 1ml per hour).

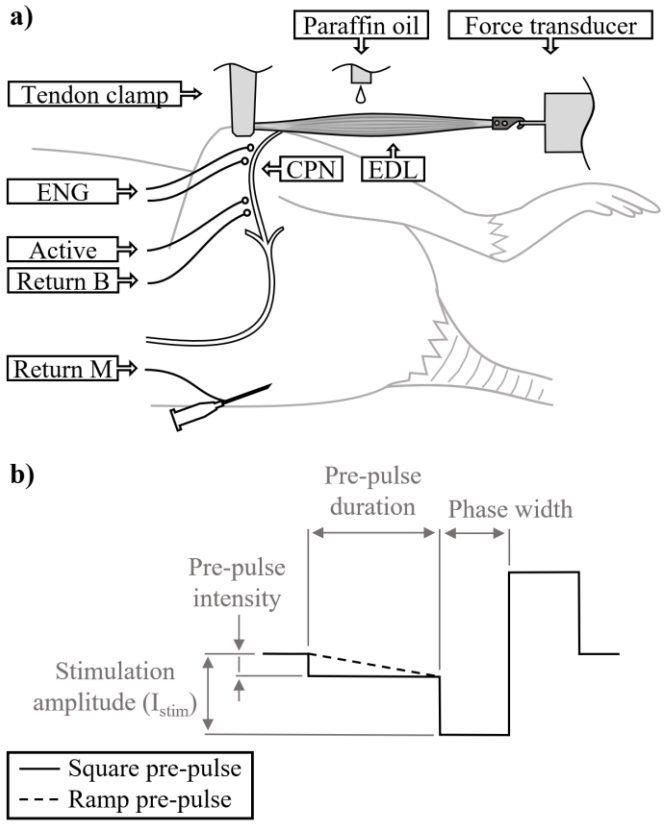


Fig. 1. Experimental model: **a)** Nerve-muscle-preparation of Common Peroneal Nerve (CPN) and Extensor Digitorum Longus (EDL). Electrodes: A pair of ENG recording electrodes (reference most distal) was placed distal to the bipolar stimulation electrode pair (active electrode more distal). For monopolar stimulation, the nerve return electrode (Return B) was disconnected and a hypodermic needle (Return M) under the dorsal skin of the animal was used as return electrode. **b)** Parameterization of pre-pulses: Square (solid line) and ramped (dashed line) pre-pulses are parameterized by pre-pulse duration of 1, 5 or 10ms and intensities of 10 or 20% of the stimulation amplitude (waveform is not to scale).

### B. Stimulation

Stimulation pulse envelopes were generated with a 1MS/s resolution using LabVIEW™ 2016 (National Instruments Corporation, Austin, Texas, U.S.A.) and sent over the analogue output of a NI PCIe 6351 Data Acquisition Card (National Instruments Corporation, Austin, Texas, U.S.A.) to a galvanically isolated voltage- to-current converter. The current-controlled stimulation pulses were delivered to the active electrode at the nerve at a rate of one pulse every 3 seconds. A relay unit was used to select under computer control between the nerve return electrode and the remote hypodermic needle return. The maximal stimulation amplitude was capped at 2mA, which was sufficient in every one of the five experiments and with every tested waveform to elicit full nerve activation.

#### 1) Subthreshold Pre-pulses

Terminology: Pre-pulses were defined by the current waveform at the active, charge injecting, electrode (Fig.2, left column). The injection of negative charge, i.e. a cathodic phase, decreases the transmembrane potential and thus depolarizes the membrane of axons near the active electrode (Fig.2 a, left). The injection of a positive, anodic charge increases the transmembrane potential at the site of injection and thus hyperpolarizes the membrane [15]. Hence a pre-pulse in the cathodic phase (at the active electrode) is referred to as a depolarizing pre-pulse (DPP) and a pre-pulse in the anodic phase as a hyperpolarizing pre-pulse (HPP).

Test pulses: All test pulses were biphasic rectangular pulses with 40µs phase width, cathodic phase first (Fig.1.b). The test pulses were delivered during four successive stimulation sessions, subdivided by pre-pulse polarity (DPPs and HPPs) and intensity (10% and 20% of subsequent test pulse). The four test stimulation sets were: 10% DPPs, 20% DPPs, 10% HPPs, and 20% HPPs. Within each of these recording sessions, the following pulse parameters were tested: Pre-pulse type (no pre-pulse, ramp, and square), pre-pulse duration (1, 5, and 10ms), and electrode configuration (monopolar and bipolar). For each parameter combination, e.g. 5ms square DPPs of 20% stimulus intensity applied via monopolar electrode configuration, a full recruitment curve in 20 steps of 50µA stimulation amplitude (typically ranging from 400-1350µA) was recorded (Fig. 3.a). All combinations of these stimulation parameters were applied in randomized order.

The upper limit of pre-pulse intensity (20% of subsequent test pulse) was chosen based on strength duration curves as a value with which no excitation was to be expected. To verify that all tested pre-pulses were indeed sub-threshold, the ENG recording of the stimulated nerve was checked. No early excitations as a response to the pre-pulse were observed, proving their amplitude never reached threshold.

Normalization: Every 20 test stimulations a standard control pulse was delivered to the monopolar electrodes. The control pulses were standard biphasic rectangular pulses with 200µs phase width, cathodic phase first, and amplitudes set separately in each experiment (typically 1mA) to elicit full nerve activation, and therefore elicit maximal isometric twitch force.

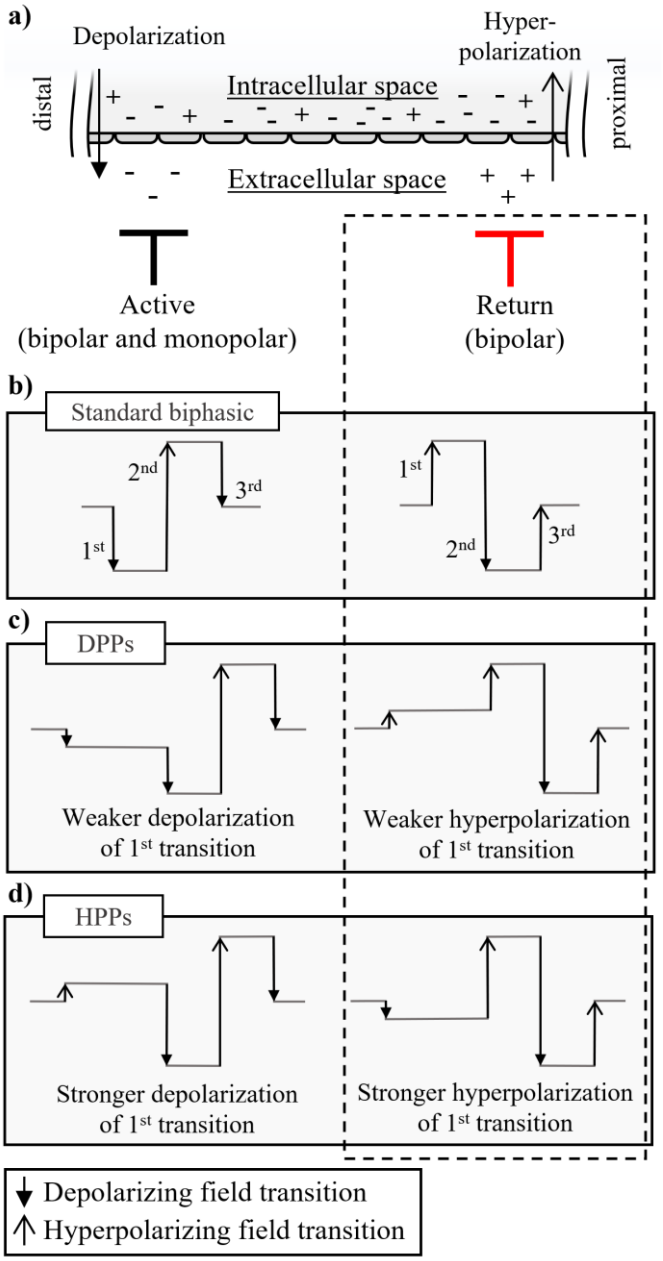


Fig. 2. Electrical fields introduced during monopolar and bipolar extracellular nerve stimulation at the active (left column) and return (right column) electrode. a) The injection of a negative or cathodic charge (electron gain) at the active electrode depolarizes the membrane of an axon near that location from its negative resting potential. The charge-return during that same stimulation (electron loss) hyperpolarizes the membrane near the return electrode for the bipolar electrode configuration. The effect of the field transitions within b) standard biphasic pulses (cathodic phase first), c) biphasic pulses with DPPs, and d) biphasic pulses with HPPs on the membrane of an axon can either be categorized as depolarizing (full arrowheads) or hyperpolarizing (open arrowheads). Waveforms not to scale.

All force responses to the test stimulations were calculated relative to the nearest control response and thus normalized. This means that recruitment curves were assembled from test pulses that were placed randomly from start to finish of the recording period, and therefore are not affected by variations of temperature, level of anaesthesia or fatigue.

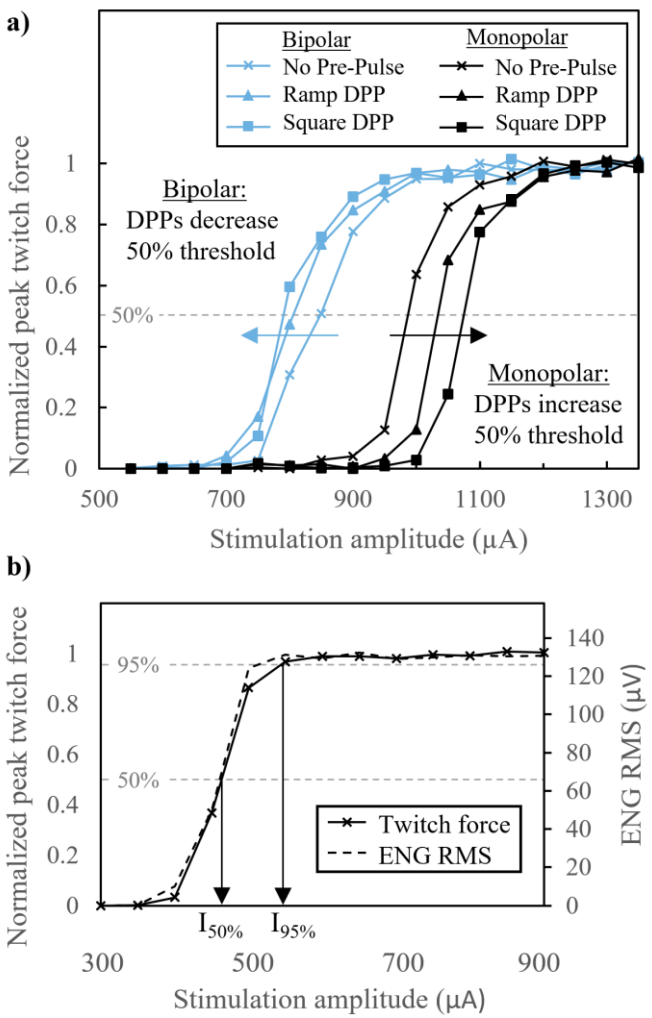


Fig. 3. **a)** Recruitment curves for 40µs biphasic stimulation with and without 5ms DPPs of 20% stimulus intensity in monopolar (black) and bipolar (blue) setup. Force responses of test stimulations were normalized to the closest control pulse (in total n = 16 control pulses (mean = 0.39N, SD = 0.01N) were used for normalization of this exemplary data set). **b)** Recruitment curve for 200µs biphasic stimulation in monopolar electrode configuration. Arrows indicate 50% and 95% thresholds ($I_{50\%}$ and $I_{95\%}$) determined by linear interpolation of normalized peak twitch force. Root mean square (RMS) of ENG recordings of the stimulated nerve correlate with isometric peak twitch force.

*2) Supramaximal amplitude stimulation*

In two animals, temporal differences in action potential generation between monopolar and bipolar electrode configurations were investigated. Amplitudes that ensured 95% neural activation ($I_{95\%}$), that is 95% of maximal isometric twitch force, were determined for phase width 100 and 200µs standard biphasic stimulations (without pre-pulses). Ten repetitions of biphasic stimulation at this 95% activation threshold $I_{95\%}$ were recorded for both phase widths and both electrode configurations. Further repeated recordings were made during stimulation at supramaximal amplitudes in 50µA increments up to $I_{95\%}$+350µA.

## C. Recording

Isometric twitch force and ENG were recorded at 100kS/sec with a PowerLab 16/35 (ADInstruments Pty Ltd, Bella Vista, New South Wales, Australia) and stored, pre-processed and exported using ADInstruments LabChart 7 Pro (ADInstruments Pty Ltd, Bella Vista, New South Wales, Australia).

## D. Data analysis and statistics

*1) Force responses*

For each tested stimulation the isometric peak twitch force response was divided by the nearest control stimulation and thus normalized. The 50% and 95% activation thresholds ($I_{50\%}$ and $I_{95\%}$) were determined for every recruitment curve by linear interpolation of the normalized experimental data points (Fig. 3). The effect of each pre-pulse on the 50% activation threshold was expressed as the percentage difference between $I_{50\%}$ without pre-pulse (i.e. standard biphasic stimulation) and $I_{50\%}$ with that pre-pulse.

Paired t-tests were performed for each pre-pulse duration (1, 5 and 10ms), polarity (DPPs and HPPs) and intensity (10 and 20%) to search for significant differences between the changes in 50% threshold in the monopolar and bipolar electrode configurations. The data for both pre-pulse shapes (square and ramp) were grouped together for these statistical tests.

*2) Electroneurography*

The ENG recordings during maximal and supramaximal amplitude stimulation were smoothed by using a 10-sample moving average (MA) filter.

# III. Results

## A. Subthreshold Pre-pulses

The dataset presented here is generated from 5900 individual responses, 1180 from each of the five experiments. The data is consistent and well-behaved (Figures 3-5) and demonstrates the value of computer-controlled experiments to extract a large amount of well controlled data from a small number of preparations, and thus achieve a reduction in the use of animals.

To ensure that all tested pre-pulses were indeed sub-threshold, the ENG recording of the CPN distal to the stimulation electrodes was checked. No early excitations as a response to the pre-pulse were observed, proving their amplitude never reached threshold.

*1) Depolarizing Pre-pulses*

DPPs increased the 50% activation thresholds in the monopolar electrode configuration and decreased stimulation thresholds in the bipolar configuration (Fig. 4). On average across subjects, DPPs (square and ramp; 1, 5, and 10ms duration) with 10% of the stimulation pulse amplitude increased thresholds in the monopolar configuration between 1.2% and 6.0% and decreased thresholds between 1.8% and 3.5% in the bipolar configuration. The difference of the effect of 10% DPPs with monopolar and bipolar electrode configuration was significant for all tested pre-pulse durations ($p<0.001$). Larger average threshold changes between 0.7% and 10.3% increase in monopolar and between 3.9% and 5.5% decrease in bipolar configurations were observed across

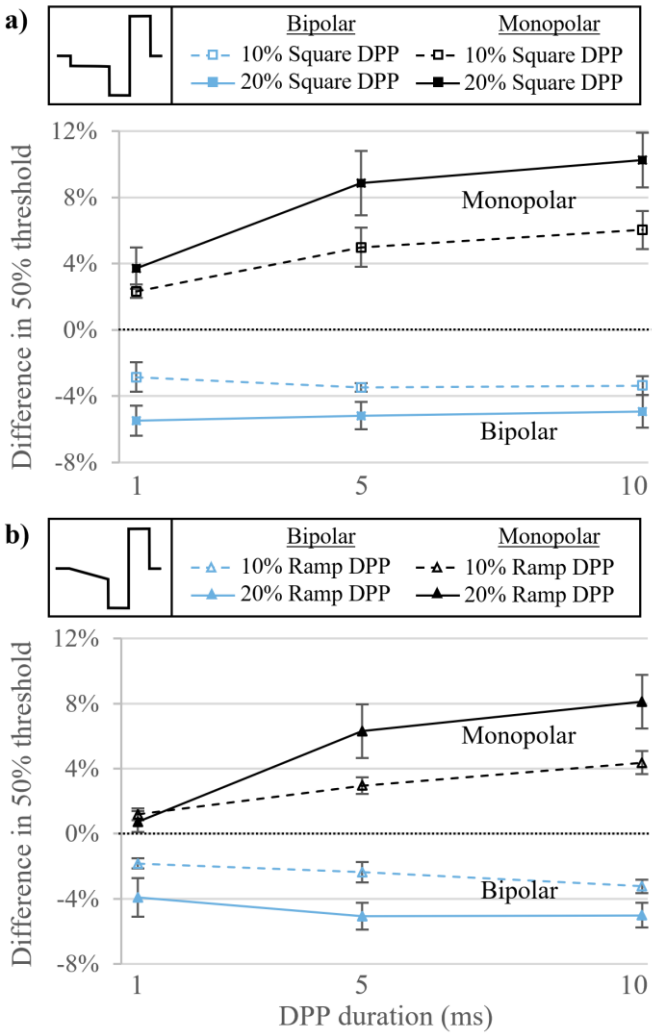


Fig. 4. Changes in 50% activation threshold with **a)** square and **b)** ramp 10% and 20% DPPs of 1, 5 and 10ms duration in monopolar (black) and bipolar (blue) setup. Negative values of "Difference in 50% threshold" indicate that the activation threshold with biphasic stimulation phase width=40µs decreased with the tested pre-pulse compared to stimulation without pre-pulse. Dashed lines represent mean ±SEM for 10% DPPs, solid lines represent mean ±SEM for 20% DPPs of n=4 animals (except for 5ms and 10ms 10% DPPs with n=5). 0% (dotted line) represents the baseline of 50% thresholds with biphasic pulses without pre-pulse. Waveforms in legend not to scale.

subjects when the DPP amplitude was set to 20% of the stimulus amplitude. For all tested pre-pulse durations, the percentile threshold changes with DPPs of 20% stimulus intensity were found to be significantly different between monopolar and bipolar electrodes (1ms: p=0.002, 5ms, and 10ms p<0.001).

*2) Hyperpolarizing Pre-pulses*

HPPs decreased stimulation thresholds in monopolar and increased thresholds in bipolar configurations (Fig. 5). The average threshold decreases across subjects with 10% HPPs (square and ramp; 1, 5, and 10ms duration) ranged from 1.7% to 3.3% for monopolar stimulation. The same pre-pulses increased thresholds on average between 0.5% and 4.1% in the bipolar case. 10% HPPs of 5ms and 10ms duration showed significant differences in the threshold changing effect between the two compared electrode configurations (p<0.01). On average across subjects, thresholds were decreased between 1.7% and 4.7% in monopolar and increased between 1.2% and 2.5% in bipolar setup, when HPPs of 20% stimulus amplitude were used. The effect of 20% HPPs was significantly different in monopolar compared to bipolar stimulation for all tested pre-pulse durations (1ms: p=0.017, 5ms: p=0.003, 10ms: p<0.001).

### *B. ENG during supramaximal amplitude stimulation*

ENG recordings of compound action potentials (CAPs) evoked by 100µs biphasic stimulation at 95% activation threshold $I_{95\%}$ had an average delay of 1.67ms ±7.2µs of the peak of the prominent first downward signal deflection from the stimulus artifact with monopolar electrodes, and an approximately one phase width greater delay of 1.78ms ±4.9µs with bipolar electrodes (Figure 6.a). Higher stimulation amplitudes led to shorter delays between artifact and ENG. In Monopolar stimulation the delay decreased by up to 60µs to 1.61ms ±6.4µs with 250µA above $I_{95\%}$. In the bipolar configuration the delay shortened by up to 170µs to 1.61ms ±6.5µs with stimulation amplitudes 300µA above $I_{95\%}$ (Figure 6.b), so that there was no difference between monopolar and bipolar.

Biphasic stimulation with 200µs phase width at 95% activation threshold $I_{95\%}$ led to a delay of 1.73ms ±6.2µs in the monopolar and 1.91ms ±4.7µs, approximately one phase width greater, in the bipolar setup (Figure 6.c). Stimulation amplitude exceeding $I_{95\%}$ led to shorter delays between artifact and signal in both electrode configurations. In the monopolar case the delay shortened up to 69µs to 1.67ms ±7.8µs at 250µA above $I_{95\%}$ and in the bipolar case greater reductions of the delay of up to 245µs to 1.67ms ±7.5µs at 350µA above $I_{95\%}$ were observed (Figure 6.d). Thereby, difference in response delay between monopolar and bipolar electrode setup diminished with increasing suprathreshold stimulation amplitude.

These are the data for one of the two rats in which ENG recordings were performed. Although absolute latency values differed, the general finding of activation one phase width later in bipolar compared to monopolar stimulation, as well as the reduction of this difference with increasing supramaximal amplitude was observed in both animals.

## IV. Discussion

Our data on the effects of DPPs and HPPs to alter the excitability of the nerve to a subsequent stimulus in monopolar electrode configuration are in good agreement with the original research by Mortimer and Grill. In their first publication on subthreshold pre-pulses, the authors used a cable model of the mammalian myelinated axon with 500µs monophasic stimuli applied via a monopolar point source. They reported increased thresholds when the stimulation pulse was preceded by 500µs DPPs of 95% threshold amplitude [6]. Further computer simulations employed a single space-clamped Node of Ranvier as well as a compartment cable model of a mammalian nerve fiber, both modelled exclusively with a monopolar stimulation point source and described threshold increases with 500µs

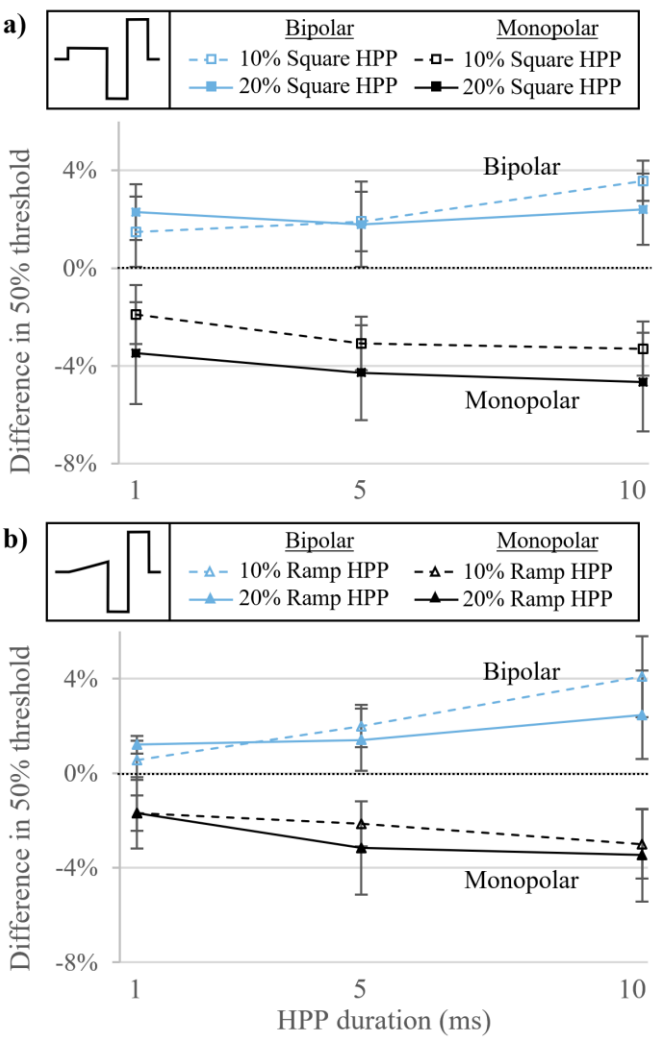


Fig. 5. Changes in 50% activation threshold with **a)** square and **b)** ramp 10% and 20% HPPs of 1, 5 and 10ms duration in monopolar (black) and bipolar (blue) setup. Negative values of "Difference in 50% threshold" indicate that the activation threshold with biphasic stimulation phase width=40µs decreased with the tested pre-pulse compared to stimulation without pre-pulse. Dashed lines represent mean ±SEM for 10% HPPs, solid lines represent mean ±SEM for 20% HPPs of n=4 animals. 0% (dotted line) represents the baseline of 50% thresholds with biphasic pulses without pre-pulse. Waveforms in legend not to scale.

DPPs of 90% threshold amplitude and threshold decreases with similarly parametrized HPPs [5]. The authors root these effects of sub-threshold pre-pulses in their influence on the inactivation variable h: DPPs decrease h and thus render the neural membrane less excitable, whereas HPPs increase h which increases the membrane excitability. In later in-vivo experiments on cat sciatic nerve, Mortimer and Grill demonstrated that 500µs DPPs of 90% threshold amplitude applied via a monopolar electrode contact selectively increased stimulation thresholds of nerve fibers that otherwise showed the lowest threshold for recruitment [7].

Using the bipolar electrode configuration that is relevant to stimulation via many nerve cuffs, electrode arrays and proposed electroceutical devices, we observed opposite effects of pre-pulses on stimulation threshold that are also in line with other published literature that does not follow the pioneer work by Grill and Mortimer [4], [11]–[13]. Most of these studies were carried out with human transcutaneous stimulation, either in a clearly bipolar configuration [11], [12] or in a monopolar setting in which the reference electrode was relatively close to the stimulated nervous structure [4].

The consistent differences of pre-pulse effects in monopolar and bipolar electrode setup in both the data described in the present study and in previously published literature, led to the hypothesis that the return electrode might be the effective electrode in bipolar stimulation setups at near threshold conditions. This hypothesis of threshold excitation at the return electrode provides a satisfying explanation for the opposite effects of pre-pulses in bipolar versus monopolar stimulation described in this study. Since the stimulation waveform is inverted at the return electrode, not only do the stimulation phases have opposite effects here but also the pre-pulses: DPPs (defined by the current waveform injected at the active electrode, compare Section II.B.1) have a hyperpolarizing effect and thus decrease thresholds in the tissue surrounding the return electrode(Fig. 2.c), whereas HPPs have depolarizing effects that render the membrane less excitable at this location (Fig. 2.d). The ENG recordings support our hypothesis by showing a shift correlating to the duration of one phase width between responses elicited by monopolar and bipolar stimulation setups at amplitudes near full activation threshold $I_{95\%}$ (Figure 6.a & 6.c). It was interpreted that while in monopolar stimulation excitation occurred near the active electrode as a response to the cathodic (first) phase (Fig.2.b, left), for the bipolar setup the return electrode was the effective electrode during the second phase, which here acts cathodically (Fig.2.b, right). In the bipolar case, the first phase effectively acts as a HPP at the return electrode and increases the resting potential of nerve fibers in proximity to this electrode. The subsequent middle field transition acts from this increased resting potential with a strong depolarizing influence on the axons in the field (compare 2$^{nd}$ field transition in Fig.2.b, right). The hypothesis is further supported by the changes in delay of elicited CAPs at supramaximal stimulation intensities (Figure 6.b & 6.d). For both tested phase widths, the reductions in the delay with increasing amplitude in monopolar setup were less than one phase width. This implies that the threshold for excitation was first reached late within the cathodic (first) phase and then gradually synchronized with the transition edge at the beginning of that phase as the amplitude increased. In bipolar stimulation however, the observed CAP delays consistently shifted more than one phase width with increasing supramaximal stimulation amplitude. This greater reduction in CAP delay supports our hypothesis that excitation begins at the return electrode during the second phase and shifts with increasing amplitude to the first phase and the more distal active electrode (compare Fig.2.b). The smooth nature of the curve of reduction of delay with increasing amplitude must represent the variation among the population of motoneurons. This provides a potent explanation for the differences in pre-pulse effects that were observed between monopolar and bipolar configurations in this study. Furthermore, the possibility of excitation at the return electrode explains work in which an "unexpected"

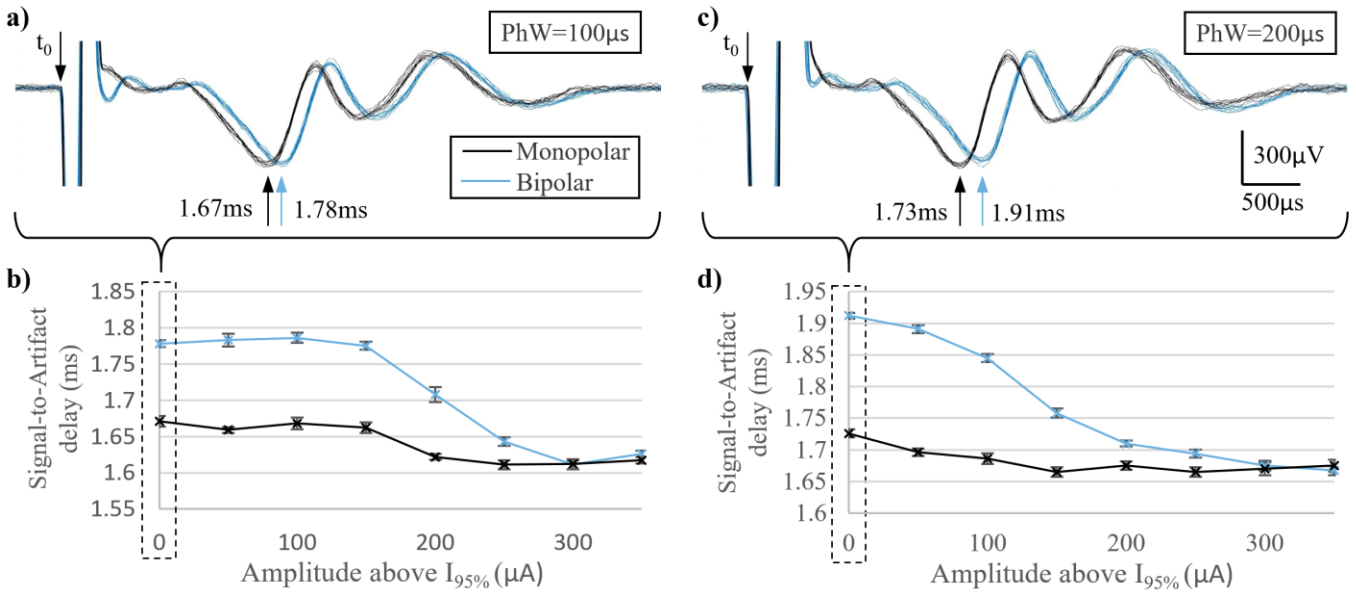


Fig. 6. Electroneurogram recordings of compound action potentials recorded in one example during standard biphasic **a)** phase width 100µs and **c)** 200µs stimulation without pre-pulse at 95% activation threshold $I_{95\%}$. Ten repeated recordings are superimposed, arrows indicate the mean delay of the first prominent downward deflection towards the stimulation artifact $t_0$. Signal-to-Artifact delays of **b)** phase width 100µs and **d)** 200µs decrease with increasing supramaximal amplitude above $I_{95\%}$. Data represents mean ±SEM of 10 repeated ENG recordings at each amplitude and phase width in monopolar (black) and bipolar (blue) electrode configuration. With phase width of 100µs (a and b) $I_{95\%}$ corresponds to 1000µA in bipolar and 750µA in monopolar configuration, for phase width of 200µs (c and d) $I_{95\%}$ corresponds to 900µA in bipolar and 600µA in monopolar configuration.

reduction of stimulation thresholds with DPPs has been reported with bipolar electrodes [11], [12].

In one experiment, 5ms and 10ms HPPs of 20% stimulation intensity led, against the generally observed trend, to lower stimulation thresholds in bipolar configuration. This caused the average threshold changes across subjects with 20% HPPs to be smaller than those observed with 10% HPPs in bipolar setup. The rationale for this might be that some aspect of the electrode configuration in this specific preparation caused the HPP to increase the stimulation thresholds at the return electrode sufficiently (and decrease thresholds at the active electrode respectively) to shift the site of AP generation from the proximal return to the distal active electrode.

Especially in the bipolar case, no significant effect of increasing the pre-pulse duration above 1ms could be observed. The rationale for this could be the separation of the pre-pulse from the effective part of stimulation (i.e. the middle field transition) by the first phase of the stimulation pulse. In this case, the pre-pulse effect might predominantly originate in a decrease (with DPPs, Fig.2.c) or increase (with HPPs, Fig.2.d) of the first pulse transition. As this transition has a hyperpolarizing effect on axons near the return electrode, a decrease of this transition edge will result in decreased thresholds for activation, as was in fact observed with DPPs in bipolar configuration. The same explanation applies to HPPs in bipolar electrode configuration: Independent from pre-pulse duration the HPPs increase the inhibitory effect of the first pulse transition (compare Fig.2.d, right) at the return electrode and thus lead to increased stimulation thresholds. The general finding of limited influence of increasing pre-pulse durations above 1ms is in agreement with the model-based work by Grill and Mortimer, who showed that increasing the DPP duration from 0.5ms to 1ms had little to no effect on the stimulation threshold of a space clamped Node of Ranvier [7]. In the same study, using a nerve cuff to stimulate the sciatic nerve in cat, the investigators described a diminishing additional effect of increasing pre-pulse duration above 5ms. All studies that tested multiple pre-pulse durations and describe a significant effect of increasing it above a duration of approximately 5ms were conducted using transcutaneous stimulation [4], [11], [12], where the tissue separating the target nerve from the electrodes is likely to influence these temporal aspects.

In general, the presented data suggests that the principal effect of any given pre-pulse may chiefly depend on the intensity and polarity of that pre-pulse at the effective location of AP generation (i.e. at the effective physical or virtual electrode). This would imply that the depolarizing effect of a sub-threshold pre-pulse at this location would also increase stimulation thresholds for other waveforms, such as monophasic or biphasic pulses with inverse phase order.

## V. Conclusion

The present study is the first to investigate the effect of DPPs and HPPs on activation thresholds with biphasic stimulation under conditions of direct relevance to contemporary miniature implantable neuromodulation devices. The significant and consistent finding of reversed pre-pulse effects in bipolar setup due to excitation at the return electrode not only helps to resolve two decades of conflicting data in the published literature on pre-pulses, but also stresses the strong influence of electrode configuration on the effect of variations in pulse shape. While the generation of action potentials at the return electrode of a bipolar electrode pair is the subject of recent research in the field of clinical nerve conduction studies [16], it has not yet

been studied in terms of its influence on the performance of implantable neuromodulation devices. A better understanding of the differences between monopolar and bipolar stimulation is of particular relevance to the emerging field of miniaturized neuromodulators, so called electroceuticals, where the small implant size does not allow for substantial separation of the electrodes. Most published data on well-established pulse shape variations such as inter phase gaps (IPGs) [17], [18], like the pioneer literature on subthreshold pre-pulses, is based on the monopolar case and therefore not directly applicable to these bipolar scenarios. We report major differences in spatial and temporal mechanisms of excitation between stimulation with monopolar and bipolar electrode positions. These findings must be taken into account when designing activation patterns delivered by miniaturized neuromodulation devices. Further investigations of action potential generation at the return electrode of a bipolar pair and its influence on the performance of implantable neuromodulators are warranted.

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