

Fibre-Selective Recording from Peripheral Nerves using a Multiple-Contact Cuff: Report on Pilot Pig Experiments

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Abstract—A single cuff electrode with multiple-contacts permits fibre selective recording from peripheral nerves. This has been demonstrated in frog nerve *in vitro* and earth worm before. In this paper we apply this method successfully to the peripheral median nerve of pig *in vivo*. Compound action potentials (CAPs) were electrically excited at the median nerve close to the wrist of the forelimb. The CAPs were recorded by a recording nerve cuff located proximal to the stimulation cuff. Applying simple mathematical routines allowed for generating a profile of nerve fibre activation as a function of propagation velocity.

I. INTRODUCTION

Erlanger and Gasser received the Nobel Prize in 1944 for their work on nerve fibre classification. They discovered the relationship between fibre diameter, action potential propagation velocity and biological function of the neural information transmitted. This concept was and still is used for diagnostic tools providing information on peripheral nerve regeneration, nerve injuries and neural diseases. Commonly, the nerve is electrically activated at one point, and the compound action potential (CAP) is recorded at two or more points along the nerve, either using nerve cuff electrodes [1] or - non invasively - surface electrodes. While this method reveals the predominant neural propagation velocity when using two or more recordings electrodes, we were interested whether this information could be extracted by using a single implanted recording cuff with multiple electrical contacts. In theory [2], this method even provides a detailed view on which fibre types actually contribute to a compound action potential. This information might be very useful for neuroscientists but also could be a valuable control for future closed-loop neural prostheses. The general feasibility of the method of velocity selective recording using a multiple-contact nerve cuff was demonstrated on frog nerve *in vitro* [3] and earthworm [4]. Here, we present the applicability to the peripheral nerve of a farm pig *in vivo*.

Manuscript received 13 April 2011.

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II. MATERIALS AND METHODS

A. Experimental Setup

A stimulation cuff electrode was used to generate electrically compound action potentials that travel along the median nerve of a pig. This neural activity was sensed by an eleven-contact nerve cuff electrode, amplified, digitized and stored for off-line data processing. During the course of the experiment the stimulation amplitude was gradually increased, leading to an increasing number of excited fibres. Applying mathematical routines, the recorded data was used to generate propagation velocity profiles, indicating which type of fibre type contributed to the compound action potential.

B. Stimulation Electrode

The stimulation cuff was fabricated in polyimide thin-film technology. This technology is described in detail elsewhere [5]. The resulting cuff consisted of two 5 μm layers of polyimide between which a 300 nm layer of platinum was sandwiched. The platinum was patterned into tracks, contact pads and electrode sites. At the locations of contact pads and electrode sites, the top layer of polyimide was removed. This structure resembles a very thin printed circuit board (PCB) rolled to form a tube with electrode contacts facing towards the lumen. After 90 min at 350 $^{\circ}\text{C}$, the tube shape became permanent, forming a cuff with an inner diameter of 1.6 mm. The contact pads were ball-bonded [5] to a ceramic PCB to which wires were soldered. The ceramic PCB including ball-bonds and solder joints was cast in rubber for electrical insulation. The stimulation cuff had three electrode contacts shaped as rings, having a contact width of 0.5 mm and a centre-to-centre distance of 5 mm; the cuff was self-curling and adapted to a nerve diameter between 1.6 and 2 mm [6]. In order to permit high electrical charges to pass through, the electrode contacts were coated with platinum black according to the recipe by Schuettler et al. [7].

C. Recording Electrode

The recording electrode consisted of a polyimide scaffold fabricated in the same technology as the stimulation cuff, carrying the electrode contacts, embedded interconnection lines and the contact pads. The scaffold was laminated between two 100 μm thin sheets of cured silicone rubber (MED-1000, NuSil, Carpinteria, Ca, USA) by plasma bonding [8]. One of the sheets had openings in the area of the polyimide scaffold electrode contacts (Fig. 1a-b). By wrapping the planar silicone-polyimide-silicone structure

around a 4 mm diameter stainless steel mandrel, a cuff was formed (Fig. 1c-d). In order to provide a reversible closing mechanism for the cuff, silicone rubber tubing of 36 mm length and 1.6 mm outer diameter was cut into segments of about 4 mm length, which were glued (MED-1000) to the cuff as shown in Fig. 1e, resulting in a piano-hinge like closure. For locking the closure, a surgical thread was pushed through the tubing segments as sketched in Fig. 1f. A cable was connected to the cuff as described in Section II-B. The recording cuff had eleven platinum electrode contacts shaped as 1 mm wide rings longitudinally distributed along the cuff with a centre-to-centre distance of 3 mm. The length of the entire cuff was 38 mm.

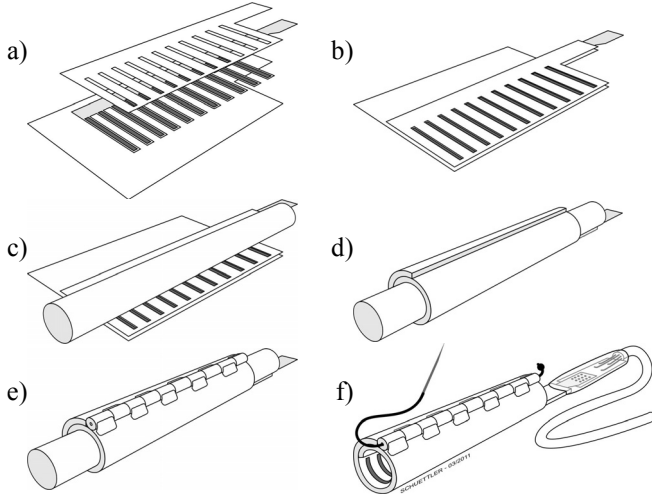


Fig. 1: A polyimide structure is sandwiched between two layers of silicone rubber (a, b) and wrapped around a mandrel (c) forming a spiral cuff (d). Silicone rubber tubing is cut into short segments, which are glued to the spiral cuff (e), resulting in a piano-hinge-closure (f).

D. Stimulator & Recording Electronics

A custom built stimulator generated a cathodic current pulse of adjustable amplitude I and 250 μs width, followed by an anodic counter pulse of $I/10$ amplitude and width of 2.5 ms. There was not interval between cathodic and anodic pulse. The stimulator provided two channels of identical output, wired to the three contacts of the tripolar cuff electrode, ensuring symmetrical flow of the current between the centre cathode and the anodes.

The recording cuff electrode was connected to a custom-built ultra low noise amplifier chip [9] that differentially amplified the voltages V_{IN} picked up by the electrodes 10,000 times and band-pass filtered it (310 Hz to 3.3 kHz). The output signals were digitized at 40 kHz/channel, 12 bit, using a commercial data acquisition system (DAQCard-6062E, National Instruments, Austin, TX, USA) controlled by a LabView program (National Instruments) running on a computer.

According to our experimental protocol, a single stimulus of given amplitude was followed by 10 ms of recording time. This was repeated 64 times at 2 Hz (limited by the recording setup) before the stimulus amplitude was changed for the

next experiment. The time between two experiments was about one minute.

E. Data Processing

Data processing was performed using Matlab software (R12, The MathWorks, Natick, MA, USA). Ten differential signals supplied by the amplifier chip were transformed to nine double-differential signals V_{OUT} (see Fig. 2), providing good rejection of signals originating from outside the nerve cuff.

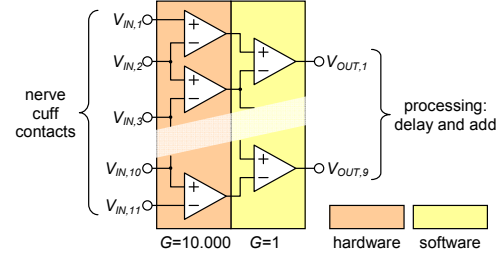


Fig. 2: Sketch of amplifying concept for extracting nine double-differential signals from eleven electrode contacts. G is the amplifier gain.

In order to extract information on the propagation velocity of the recorded CAPs travelling along the nerve, the double-differential signals V_{OUT} were delayed relative to each other, e.g.: $V_{OUT,2}$ was delayed by dt against $V_{OUT,1}$. $V_{OUT,3}$ was delayed by $2 \cdot dt$ relative to $V_{OUT,1}$, etc. After delaying, all nine signals were combined to one signal V_{DS} by summation in the time domain according to Eq. 1.

$$V_{DS}(t, dt) = \sum_{i=1}^9 V_{OUT,i}(t - (i-1) \cdot dt) \quad \text{Eq. 1}$$

A delay profile was calculated by determining the maximum voltage value $V_{MAX}(dt)$ of V_{DS} for each dt . The propagation velocity v equals p / dt , with $p = 3$ mm being the pitch of two adjacent electrode contacts. This allowed for the calculation of a velocity profile $V_{MAX}(v)$. Defined by the sampling frequency of 40 kHz, dt is 25 μs or its multiples, providing a rather low resolution of the profile in the high velocity range: $v = 120$ m/s, 60 m/s, 30 m/s, etc. In order to increase the resolution, the voltages V_{OUT} were interpolated to a virtual sampling frequency of 200 kHz. Now, dt became 5 μs or its multiples and the resolution in the velocity domain was increased accordingly (in the range $v \leq 120$ m/s: 120 m/s, 100 m/s, 86 m/s, 75 m/s, 67 m/s, 60 m/s, etc.).

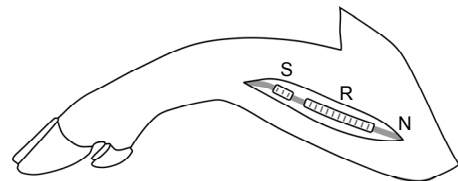


Fig. 3: Location of stimulation (S) and recording (R) cuff electrodes at the median nerve (N) in pig forelimb.

F. Surgical Procedure

A female adolescent (50 kg) farm pig was anaesthetized using Isofluran and Dormicum, intubated and the medial nerve of the right forelimb was exposed over a length of about 80 mm. The recording nerve cuff was implanted close to the elbow joint. Distally on the same nerve, a tripolar stimulation cuff was placed. The location of the implantation site is indicated in Fig. 3, the distance between the two cuffs was about 40 mm. All surgical procedures were carried out according to the guidelines for ethical treatment of animals of the university hospitals of Aarhus, Denmark.

III. RESULTS

A. Electrodes

The stimulation cuff is a self-spiralling design that adapts to any diameter between 1.5 mm and 2.0 mm in diameter. Fig. 4 shows a photo of the recording cuff, closed and locked by a blue thread.

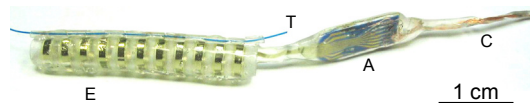


Fig. 4: Photograph of multiple-contact recording cuff (E), closed with a blue surgical thread (T), connected to an alumina substrate (A) to which a cable (C) is soldered.

B. Surgical Procedure

During the surgical procedure, we observed some nerve swelling in the distal region of the exposed nerve (Fig. 5) to up to ~6 mm diameter. This large diameter prohibited the stimulation cuff from being completely wrapped around the nerve. The electrode contacts touched only one half of the nerve circumference. In the proximal region, the nerve diameter did not change, permitting the 4 mm recording cuff to be implanted, closed and locked by a surgical thread without difficulties. Saline solution was injected into the cuff in order to displace any air bubbles, ensuring a good electrical contact between electrodes and nerve.

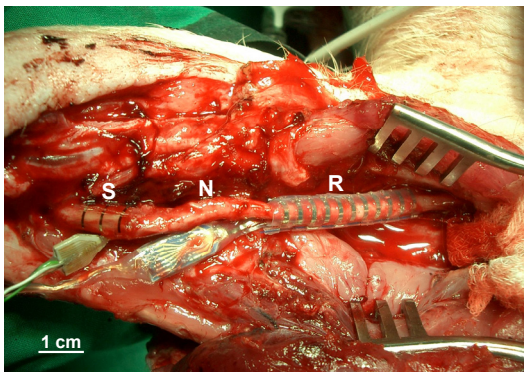


Fig. 5: Photograph of stimulation (S) and recording (R) cuff implanted on pig median nerve (N).

C. Data Processing

The recording of the first 5 ms of an experiment is shown in Fig. 6 and Fig. 7. Beginning at a very low stimulation amplitude and gradually increasing it, we found 200 μA to be the threshold for generating CAPs (Fig. 6). Depending on the tripole, we recorded voltages between 5.13 and 18.3 μV_{pp} while the noise floor was calculated to range from 0.982 to 2.73 μV_{rms} based on the last half of the 10 ms recording of each channel, which is discarded in the figure. Manual estimation of the propagation velocity was carried out by measuring the delay between clearly-visible peaks of different channels, e.g. the delay between peaks from channel one and channel eight is $dt = 0.35$ ms. The distance from tripole one to tripole eight was $x = 21$ mm, therefore the velocity is $v_F = x / dt = 60$ m/s.

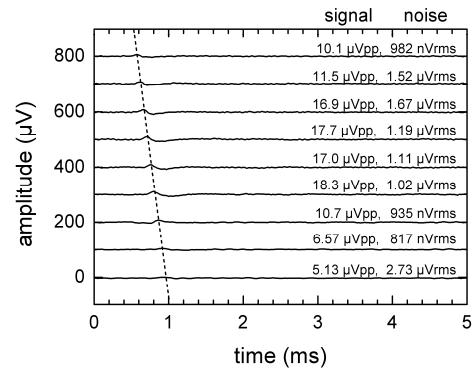


Fig. 6: Recorded CAPs after double-differential amplification (V_{OUT}). The dashed line indicates the propagation of positive peaks from the first tripole (top) to the ninth tripole (bottom). Stimulus amplitude: 200 μA .

With increasing stimulation amplitude, the CAP amplitudes increased. At 4 mA the excited CAPs had peak-to-peak voltages between 56 and 157 μV_{pp} (Fig. 7). The noise was found to be between 0.784 and 2.57 μV_{rms} . The delay between the first and the ninth fast peak was 0.4 ms. This corresponds to a velocity of $v_F = 24$ mm / 0.4 ms = 60 m/s. Additionally, a second positive phase became visible. However, this positive peak was less sharp. A time delay between the first and the fourth tripole was estimated to be 0.21 ms, suggesting a propagation velocity of $v_S = 43$ m/s.

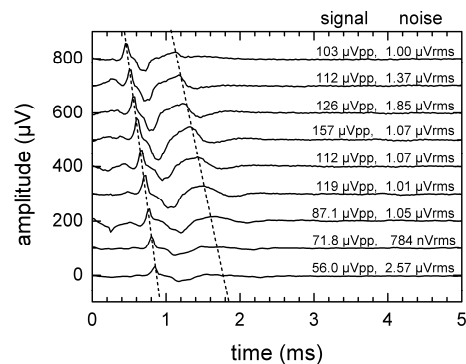


Fig. 7: Recorded CAPs after double-differential amplification (V_{OUT}). The dashed lines indicate the propagation of positive peaks from the first tripole (top) to the ninth tripole (bottom). Stimulus amplitude: 4 mA.

Applying the algorithm for obtaining a velocity profile led to Fig. 8. Although we repeated a stimulus 64 times, the recordings following a single stimulus were sufficient to allow the calculation of a velocity profile, so we omitted the option of averaging the CAP recordings. With rising stimulus amplitude, an increasing number of fast fibres ($v_F = 60$ m/s) was activated. At 4 mA stimulus amplitude, a slower fibre population ($v_S = \sim 30$ m/s) contributed to the CAP.

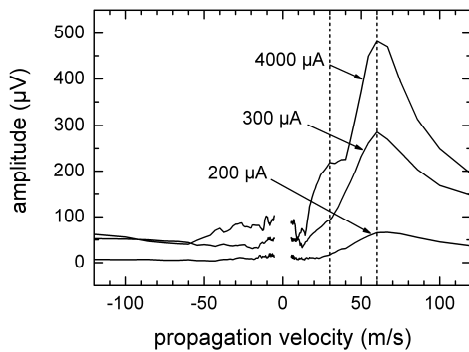


Fig. 8: Velocity spectra obtained by three successive experiments. The vertical dashed lines indicate positive velocities at 30 m/s and 60 m/s, respectively. No averaging was applied to obtain this velocity profiles. Positive velocities correspond to CAPs travelling in proximal (afferent) direction, negative velocities indicate efferent activity.

IV. DISCUSSION

We were able to obtain velocity profiles from electrically evoked compound action potentials in pig peripheral nerve, although the experimental setup offered room for improvement, e.g. the swelling of the nerve could be minimized in future with the experience gained during the first surgery. The stimulation cuff was too small for the nerve, even before swelling occurred.

There was no stimulus artefact in the recording, most likely due to the long distance between the stimulation and recording site. However, the recording amplitudes of the CAP varied along the cuff. Especially at the proximal end, where the amplitudes are halved compared to the distal end. A possible explanation is a re-arrangement of the stimulated nerve fibres inside the nerve, changing from superficial location (close to the electrode contacts) to a deeper location in the nerve tissue, having inactive nerve fibres attenuating their action potentials. A variation in the contact between the entire nerve and the cuff electrodes by connective tissue or nerve diameter variations are also potential causes for the differences in recorded amplitudes.

No averaging was required to obtain very clear signals with good signal to noise ratio. This can be attributed to the excellent noise characteristics of the purpose-built amplifier chip as well as the double-differential amplification scheme, which also reduced the stimulation artefacts to negligible levels. The signal to noise ratio is inherently enhanced by the methods for generating velocity profiles since the amplitudes of the nine recordings are added-up constructively while the random noise floor tends to cancel itself out.

The detection of the propagation velocity of the slow fibre population was difficult, because the fast and the slow activity seem to melt into each other in the velocity profile (Fig. 8). It is unclear whether the manual method ($v_S = 43$ m/s) or the profile ($v_S = \sim 30$ m/s) provides a more accurate value. In order to address this problem, the method requires a tool for increasing the velocity selectivity. We show elsewhere that band-pass filtering in the frequency domain is a simple and effective tool for improving the selectivity of the method of multiple-contact cuff recordings [10].

V. CONCLUSION

The method of velocity selective recording using a single multi-contact nerve cuff was successfully applied to the peripheral nerve of a pig *in-vivo*. A single electrical stimulus was sufficient to obtain a velocity profile, indicating the propagation velocities of the predominant fibres contributing to an electrically evoked compound action potential. This method might have many applications as a tool in fundamental neuroscience and could possibly provide selective information on neural activity as a control for neural prosthesis. However, for the latter, it remains to be proven that the method is applicable to physiological nerve activity.

ACKNOWLEDGEMENTS

This work was supported in part by the German Academic Exchange Service (DAAD) and by the Engineering and Physical Sciences Research Council (EPSRC), UK.

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