P2 - Poster Session

In vitro Large Polyfascicular Nerve Model for Assessment of Fascicular Recruitment Characteristics of Peripheral Nerve Interfaces*

Kristian R. Harreby, Cristian Sevcencu, and Winnie Jensen

Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology (HST), Aalborg University (AAU), Denmark
{Krauhe,wj}@hst.aau.dk, sevcr@hstaau.dk

Abstract. The use of neuroprosthetic devices is currently hindered by a lack of safe and selective peripheral nerve interfaces (PNI). Research in the area typically characterizes new PNI designs in small simple nerve models using indirectly parameters of nerve recruitment.

The current work presents a new human size polyfascicular in vitro nerve model which enables direct assessment of fascicular recruitment characteristics of PNIs.

The model was used for characterizing the transverse intrafascicular multichannel electrode (TIME). Preliminary results demonstrate the feasibility of using the model and illustrate the high detail level which can be obtained with this model compared to typically used in vivo nerve models

1 Introduction

In spite of extensive studies on PNIs for selective activation, there are only few commercially available neural stimulation devices, most of which utilizes non-selective recruitment of peripheral nerves [1],[2]. Advanced neuroprosthetic applications would be possible if selective, safe and easy to implant PNIs were developed.

New PNI designs are typically tested in *in vivo* nerve models using small mammals such as rat, cat and dog [3],[4] where the recruitment of skeletal muscles are used for assessment of the nerve recruitment characteristics. The nerves of these animals are, however, much smaller and simpler (fewer fascicles) than human nerves [5]. In addition, muscle derived parameters only provide indirectly information about the actual recruitment of motor fibers.

* This work was funded by the European Union in the 7th framework program under Grant CP-FP-INFSO-224012/TIME. The transverse intrafasicular multichannel electrodes (TIME) were provided by Tim Boretius and Thomas Stieglitz, Department of Microsystems Engineering, University of Freiburg, Germany.

J.L. Pons et al. (Eds.): *Converging Clinical & Engi. Research on NR*, BIOSYSROB 1, pp. 403–407.
DOI: 10.1007/978-3-642-34546-3_65

This work describes a new *in vitro* nerve model which enables direct assessment of the recruitment of individual fascicles in human size polyfascicular nerves. As an example the fascicular recruitment of the TIME is assessed in the median nerve of a pig.

2 Methods

2.1 Preparation

Immediately after the euthanasiation of a farm pig (weight ~20 kg) access to the median nerve was provided through the axilla. The nerve was freed before cutting it proximal and distal (length ~10 cm, explant time 10-15 min) and placed on a sheet of Sylgard in a dish with Ringer solution (NaCl: 146 mM/l, KCl: 5 mM/l, MgCl2: 1 mM/l, CaCl2: 2 mM/l, HEPES: 10 mM/l, Glucose: 11mM/l, PH adjusted to 7.4 using NaOH, room temperature). The solution was periodically bubbled with O_2.

At the distal end of the nerve fascicles were freed and a tungsten needle electrode (100 µm diameter) was inserted into each fascicle. A silicone tube (cut in half in the longitudinal direction, internal diameter 0.25 mm) was mounted on each needle. When the needle was inserted the tube isolated the fascicle and electrode from the solution.

At the proximal end, a bipolar cuff electrode (contact spacing 3 mm, diameter 4.0 mm) was mounted around the nerve. The TIME was used as an example of a PNI in this study. This PNI has 6 iridium oxide coated contact sites (pitch 440 µm) on each side of a flexible polyimide loop (referred to as 1-6 and 1'-6') which was pulled transversely through the nerve [6]. Two TIMEs were implanted, transversely through the nerve just distally to the cuff.

To estimate 100 % recruitment, cuff stimulation was performed (charge balanced pulses up 1.60 mA, duration of 300 µs, delivered at 4.76 Hz) while recording (sampling frequency = 20 kHz) the evoked electrofasciculogram (EFG) of individual fascicles. Monopolar cathodal stimulation (charge balanced, fixed 1.14 mA pulses, duration 100 µs, delivered at 4.76 Hz) was then delivered through the TIMEs contact sites (a stain less steel electrode in the solution functioned as anode).

Explantation, preparation and experimental procedures took around 5 hours.

2.2 Offline Analysis

During offline EFG assessment stimulation artifacts were blanked out, the signal was band pass filtered (0.1- 2 kHz), and responses corresponding to the same stimulation intensities were synchronized averaged. Finally the peak-to-peak voltage (Vpp, in the 2 – 5 ms interval following stimulation pulse onset) was used as a measure of recruitment. The highest Vpp obtained during cuff stimulation was used for defining the 100 % recruitment level of each fascicle.

3 Results

EFG was obtained from 23 fascicles. Recording was not possible from 3-5 fascicles due to space limitations.

Evoked responses lasted from 1.5 ms up to 5 ms after stimulation pulses were initiated, see Fig.1 (a). Maximal evoked Vpp reached was 38.8 ± 45.6 µV (mean ± SD) across all EFG channels.

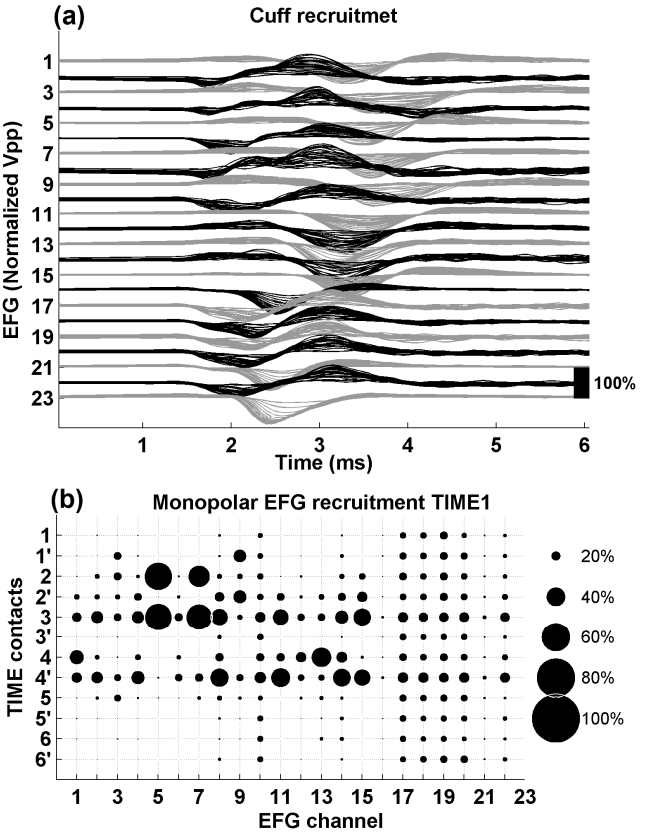

Fig. 1 (a) Gradual increasing EFG responses observed when stimulating with the cuff electrode (800-1600 µA with increments of 40 µA). Stimulation pulses start at t = 0 ms. (b) Recruitment level of individual fascicles when applying stimulation at the individual contact sites of the TIME

The cuff evoked responses in all channels from when stimulating from 800 µA to 1600 µA (pulse duration: 300 µs), however, most fascicles did not display a plateau in recruitment curves. Ideally, plateaus should have been reached to estimate the maximal recruitment level.

The TIMEs started to evoke EFG responses only when close to their charge capacity limit (1200 nC). Therefore the stimulation was assessed only for a fixed stimulation intensity (current: 1140 μA, duration: 100 μs, corresponding to 95% of maximal charge capacity).

Both TIMEs evoked responses in several fascicles. Some fascicles were selectively recruited by only a few contact sites e.g. fascicle 5 and 7 in Fig. 1 (a), whereas other fascicles seemed always to display some recruitment independently of contact sites (e.g. fascicles 17 to 23).

4 Discussion

Compared to our *in vivo* experiments in pigs [7] the recruitment threshold was significantly elevated in the *in vitro* model. This might be due to the temperature (~22ºC) under which the experiment was conducted or due to the relative long preparation and experimental time needed.

Better quality EFG might be obtained using more advanced fascicle electrodes [8]. However, because of the high number of fascicles and their close proximity small simple needle electrodes were preferred.

The results show the feasibility of assessing nerve fascicle recruitment directly and in much more detailed than in *in vivo* models using muscle derived parameters. In principle, any PNI which is to be implanted on or in the nerve can be characterized on/in the most relevant animal nerve in relation to the target human neuroprosthetic application. For example if the TIME was to be used for inducing sensory feedback in the median nerve of an amputee, the number of significant different recruitment patterns would give an indication of how many degrees of freedom of sensory inputs could be produced. In Fig. 1 b, contact sites 1',2,2',4,4' seems to provide different recruitment patterns, indicating that an amputee implanted user may be able to distinguish between stimulation performed at these contact sites.

In *in vivo* pig studies we have characterized the TIME induced recruitment of the median nerve via the electromyogram from 7 muscles [7]. Using the *in vitro* model the activation of the same nerve could be characterized in further details via the 23 EFG channels. The detail level would have been even higher if all fascicles in the nerve had been monitored (typically 29 ± 10 [5]).

5 Conclusion

These preliminary results indicate the feasibility of assessing the recruitment of PNIs in large human size nerves to an unprecedented detail level. If this *in vitro* model is further combined with histology analysis and a computer nerve model this may constitute a strong and flexible platform for test and development of future PNIs.

References

[1] Burridge, J.H., Haugland, M., Larsen, B., Pickering, R.M., Svaneborg, N., Iversen, H.K., et al.: Phase II trial to evaluate the ActiGait implanted drop-foot stimulator in established hemiplegia. J. Rehabil. Med. 39, 212–218 (2007)

[2] De Herdt, V., Boon, P., Ceulemans, B., Hauman, H., Lagae, L., Legros, B., et al.: Vagus nerve stimulation for refractory epilepsy: a Belgian multicenter study. Eur. J. Paediatr. Neurol. 11, 261–269 (2007)

[3] Yoo, P.B., Sahin, M., Durand, D.M.: Selective stimulation of the canine hypoglossal nerve using a multi-contact cuff electrode. Ann. Biomed. Eng. 32, 511–519 (2004)

[4] Branner, A., Stein, R.B., Fernandez, E., Aoyagi, Y., Normann, R.A.: Long-term stimulation and recording with a penetrating microelectrode array in cat sciatic nerve. IEEE Trans. Biomed. Eng. 51, 146–157 (2004)

[5] Kundu, A., Harreby, K.R., Jensen, W.: Comparison of median and ulnar nerve morphology of Yorkshire landrace pigs and Göttingen mini pigs. In: The IFESS Conf. (to be published, 2012)

[6] Boretius, T., Yoshida, K., Badia, J., Harreby, K.R., Kundu, A., Navarro, X., et al.: A Transverse Intrafascicular Multichannel Electrode (TIME) to Treat Phantom Limb Pain – Towards Human Clinical Trials. In: IEEE Int. Conf. on Biomedical Robotics and Biomechatronics (to be published, 2012)

[7] Kundu, A., Harreby, K.R., Kurstjens, M., Boretius, T., Stieglitz, T., Yoshida, K., Jensen, M.: Comparison of acute stimulation selectivity of transverse and longitudinal intrafascicular electrodes in pigs. Annu. Me. of the Soc. for Neuroscience No. 495.07/SS31 (2011)

[8] Floreu, E., Kriebel, M.E.: A new suction-electrode system. Comp. Biochem. Physiol. 18, 175 (1966)