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Published in:
Direct Nerve Stimulation for Induction of Sensation and Treatment of Phantom Limb Pain

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

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Selectivity of the TIME Implantable Nerve Electrode

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6.1 Introduction

For restoring the lost function of organs innervated by damaged peripheral nerves or to control a prosthesis substituting a lost organ, it is necessary to provide an array of electrodes with good capabilities for both stimulation and recording neural activity. Ideally, a bidirectional interface for the control of a bionic prostheses in amputees should allow on the one hand, recording of neural efferent motor signals to be used for the motion control of the mechanical prosthesis, and on the other, stimulating afferent sensory nerve

fibers within the residual limb to provide sensory feedback to the user from tactile and force sensors embedded in the prosthesis. In amputees, the nerves in the stump, which previously innervated the missing limb, are still functional, even years after the amputation has occurred (Dhillon et al., 2005). If these nerves could be selectively interfaced, it would be possible to translate the motor signals into adequate actions and to provide the amputated subject with sensory input, perceived as originating from the missing limb.

The selection of a suitable nerve electrode has to consider a balance between invasiveness and selectivity, with the ultimate goal of achieving the highest selectivity for a high number of nerve fascicles by the least invasiveness and potential damage to the nerve. For controlling an advanced neuroprosthesis, multiple functions, either motor or sensory, conveyed by separate fascicles or bundles of axons in a peripheral nerve have to be interfaced. To adequately reproduce motor and sensory functions, it is, however, necessary that the interface achieves two conditions: topographical selectivity and functional selectivity. The knowledge of the fascicular topographical pattern of peripheral nerves of interest (Gustafson et al., 2009; Badia et al., 2010; Delgado-Martinez et al., 2016) will improve the implantation of electrodes in adequate positions, by placing at least one active site within each fascicle of interest. Regarding functional selectivity, for example, to induce a particular sensory input, e.g., touch sensation from the thumb, it is needed to selectively activate the specific nerve fibers that before amputation mediated touch information from the thumb to the brain. This should be done without recruiting fibers mediating information related to other sensory modalities or limb areas, which might overshadow the intended sensory input. The more individual parts of the nerve that can be selectively interfaced without recruiting other parts, the higher chance is that a specific part of the nerve can be targeted in a functional relevant way and the more selective the interface is. An intrinsic drawback, however, is that the invasiveness and the risk of the surgical implantation procedure tend to increase the more contact sites are placed in or around a nerve and the closer they are placed to individual nerve fibers.

The design of the transversal intrafascicular multichannel electrode (TIME) aimed to maximize the number of contact sites and their proximity to different populations of nerve fibers, while keeping the risk and invasiveness of the implantation low. The TIME is intended to be implanted transversally in the nerve and cross several fascicles or subgroups of nerve fibers with one single device (Boretius et al., 2010). By placing a number of active sites distributed along the intraneural implant, a single TIME is thus able to interface several fascicles, reducing the number of implanted devices with respect to other intrafascicular electrodes.

The TIME was extensively tested in computer simulations and animal studies. These studies had several purposes: (1) to develop and test surgical techniques, (2) to ensure the durability and biocompatibility of the electrode when exposed to the biological environment (see Chapter 5), (3) to evaluate the selectivity performance for stimulation and recording neural activity, and (4) to evaluate the performance over time. The current chapter focuses on the two last objectives.

6.2 Evaluation of TIME in the Rat Sciatic Nerve Model

6.2.1 Stimulation Selectivity

The testing in preclinical animal models is obliged before translation into human subjects to avoid any risk, and also to obtain adequate information regarding implantation and performance of the electrode. However, one disadvantage is that it is not possible to get a subjective description of the percepts induced by activating the nerve in these models, and thus the selectivity performance of the implanted electrode must be quantified by neurophysiological methods. In the following studies, for practicality, the tests for selectivity were performed in an inverse way to what it is pretended in human amputees, i.e., selectivity of stimulation of motor nerve fibers, and selectivity of recording of sensory nerve fibers.

6.2.1.1 Methods

Acute experiments were made on adult rats in which the sciatic nerve was implanted with an electrode for assessing selective electrical stimulation at the fascicular and subfascicular levels. We compared the results obtained with three types of neural electrodes: TIME, LIFE, and cuff (Badia et al., 2011). The three types of electrodes had adequate dimensions for the rat sciatic nerve and were made on polyimide substrate with platinum active sites and contact lines. In one group of rats (group TIME-A) the TIME (Boretius et al., 2010) was transversally implanted traversing the tibial and peroneal fascicles of the sciatic nerve. With the aim of assessing the selectivity of TIME just in the tibial nerve (subfascicular selectivity) the TIME was implanted in dorsoventral direction across the tibial nerve (group TIME-B). To compare the results of TIME with Cuff and tf-LIFE devices, cuff electrodes of 12 poles (four tripoles) (Navarro et al., 2001) were implanted around the sciatic nerve (group Cuff), and tf-LIFEs (Lago et al., 2007) were implanted longitudinally in the tibial nerve (group LIFE).

Stimulation was provided by a STIM'3D stimulator (Andreu et al., 2009), delivering series of monophasic rectangular pulses of 10 μs at 0.5 Hz, increasing the intensity in steps of 20 μA from 20 to 300 μA for TIME and LIFE and to 800 μA for cuff electrodes. Pulses were delivered through each of the active sites of the TIME and tf-LIFE against a small needle electrode placed near the nerve. For the cuff, stimulation was applied to each one of the four tripoles in the cuff, using the central pole as cathode and the outer poles as anodes.

The activation of tibialis anterior (TA), gastrocnemius medialis (GM), and plantar interosseus (PL) muscles was studied to verify if it was possible to stimulate one of the three muscles without producing significant excitation of the other two. The fibers innervating the TA muscle are located in the peroneal branch, whereas those innervating the GM and the PL muscles are in distinct locations in the tibial branch (Badia et al., 2010). The compound muscle action potentials (CMAPs) were recorded by small needle electrodes placed in each muscle, amplified and filtered (5 Hz, 2 kHz). The amplitude of the CMAP (M wave) was normalized to the maximum CMAP amplitude obtained for each muscle in the experiment. For each active site (as), k, a selectivity index (SIas) was calculated as the ratio between the normalized CMAP amplitude of that muscle, CMAPni, and the sum of the normalized CMAP amplitudes elicited in the three muscles (Veraart et al., 1993):

$$SIas_{i,k} = \frac{CMAPni}{\sum_j CMAPnj} \dots \tag{6.1}$$

this index ranges from 0 (no activation of the target muscle) to 1 (activation of only the target muscle).

In order to compare the stimulation selectivity of the three devices, a selectivity index of the device (SId) was calculated. The SId was the product of the highest SIas for each muscle with one given electrode, and may range from 0.0307 (no selectivity; each $\mathrm{SIas} = 0.333$ for three muscles considered) to a maximal of 1.0 (maximal selectivity; each $\mathrm{SIas} = 1$).

$$SId = SI_{asPl} \times SI_{asGM} \times SI_{asTA} \dots \tag{6.2}$$

6.2.1.2 Results

At low stimulation intensity selective stimulation of one muscle could be detected, due to the close contact of one electrode site with the corresponding muscular nerve fascicle. By progressively increasing the intensity of the

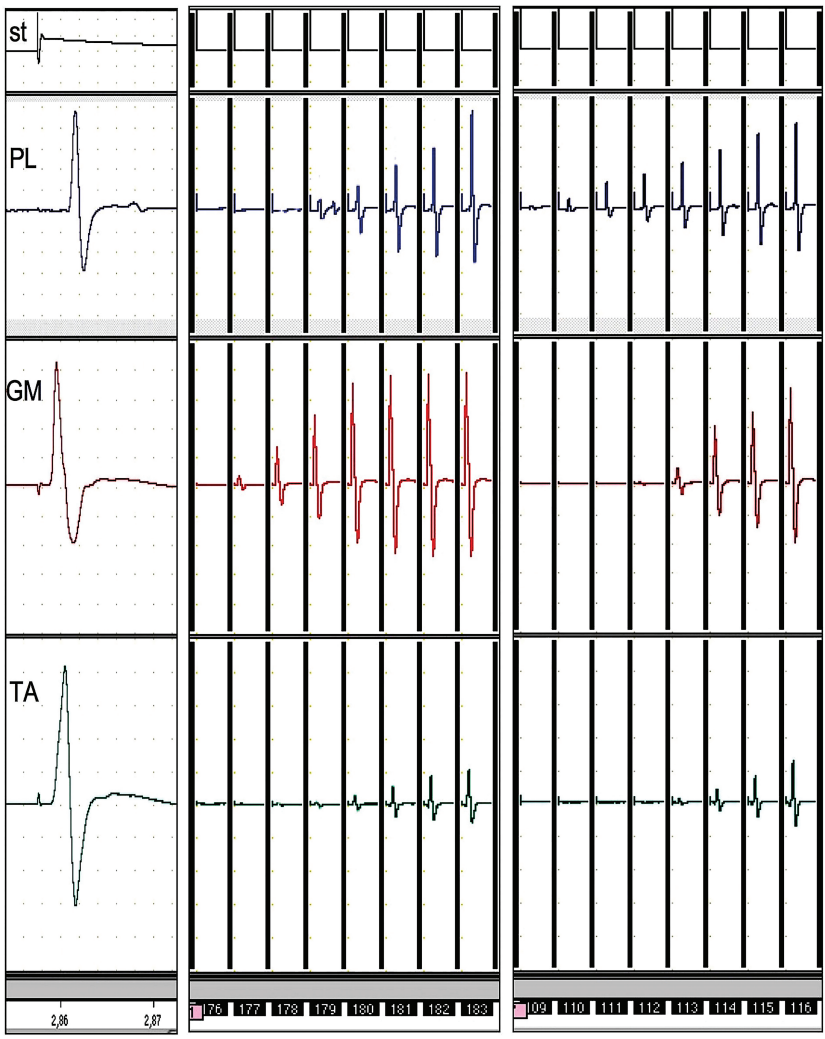

Figure 6.1 Examples of CMAPs recorded in plantar (PL), gastrocnemius medialis (GM), and tibialis anterior (TA) muscles with stimulation (st) at increasing pulse intensity, delivered from two different active sites of a TIME implanted in the rat sciatic nerve. Selective activation of GM (center block) and selective activation of PL muscle (right block) can be observed.

stimulus, the amplitude of CMAPs increased to reach the maximal value (i.e., activation of the whole muscle); however, the stimulus spread to other surrounding fascicles, therefore activating two or the three muscles tested (Figure 6.1).

The TIME device allowed selective activation of the PL, GM, and TA muscles when stimulating through different active sites. Selective activation using a multipolar cuff electrode was only possible for two muscles, GM and TA, whereas with the tf-LIFE it was not possible to activate selectively more than one muscle (either PL or GM for the implants in the tibial nerve). The intrafascicular electrodes TIME or LIFE provided excitation limited to the implanted fascicle, but did not extend stimulation to other nearby fascicles crossing the perineurial barrier (Badia et al., 2011).

The threshold for muscle activation was found at intensities between 24 and 66 μA similar for TIME and LIFE devices, and at higher levels ranging 180–330 μA with cuff electrodes. The mean thresholds with the TIME did not differ significantly between the three muscles. Cuff minimum threshold for the PL muscle was significantly higher than the threshold for GM and TA muscles, likely indicating a deep vs. superficial location of the corresponding motor fibers within the nerve.

With the LIFE implanted in the tibial fascicle good selectivity was obtained for the GM, moderately for the PL, but no activation of the TA muscle was possible. With the cuff electrode good selectivity was found for the GM and TA muscles, i.e., between different fascicles in the same nerve trunk, but it was not possible to separately activate PL from GM muscle, at the subfascicular level. In contrast, with the TIME different active sites selectively activated each one of the three muscles evaluated, indicating potential selectivity at fascicular and subfascicular levels (Figure 6.2). Calculation of the SId showed that the TIME has higher selectivity in the three muscles model tested than the cuff and LIFE electrodes.

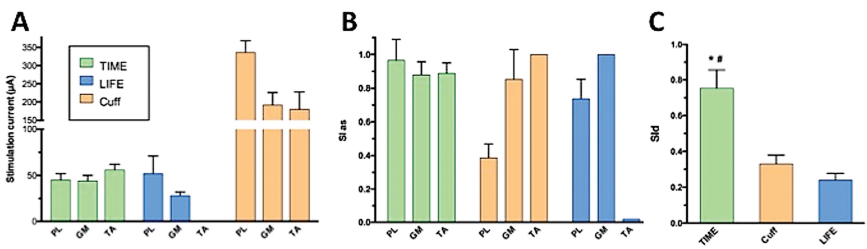

Figure 6.2 (A) Plot of the threshold of activation of the motor fascicles innervating PL, GM, and TA muscles, with TIME, LIFE, and cuff electrodes. (B) Plot of the Sias (best active site in each electrode) obtained for each of the muscles tested with TIME, LIFE, and cuff electrodes. (C) Plot of the SId corresponding to TIME, Cuff, and LIFE devices. Bars are mean and SEM. * $p < 0.05$ vs. Cuff; # $p < 0.01$ vs. LIFE. Data from Badia et al., 2011.

In order to evaluate the changes in selectivity depending on the relative location of the implanted TIME within the sciatic nerve, after performing the stimulation protocol with one TIME, we moved the TIME a distance of about 0.1 mm in the same direction that it was implanted and repeated the stimulation protocol. The comparison of the recruitment curves and the SIas showed variations as high as 100% in the SI for individual active sites between the two trials. This observation is a proof of concept that, once implanted, the intraneural TIME can be carefully repositioned to obtain the optimal stimulation selectivity of different fascicles and subfascicles in the nerve.

6.2.2 Recording Selectivity

To verify that the TIME was able also to serve as an adequate bidirectional interface, acute experiments were made on adult rats in which the sciatic nerve was implanted with a TIME for assessing its properties for recording neural activity. The aims of this work were: (1) to characterize the recording capabilities of the TIME in an experimental model, (2) to assess the spatial selectivity of the TIME for recording sensory neural activity at the subfascicular level, and (3) to assess the potential of TIME-recorded signals for discriminating neural activities evoked by stimuli of different sensory modalities (Badia et al., 2016).

6.2.2.1 Methods

The TIME was transversally inserted across the two main fascicles of the sciatic nerve, the tibial and peroneal branches, proximal to the knee, as reported in the previous section. The insertion was monitored under a dissection microscope to ensure that the TIME active sites were located as: the first three of the right and left branches inside the tibial nerve, and the fourth ones within the peroneal nerve (Figure 6.3).

For assessing the spatial selectivity, compound nerve action potentials (CNAPs) were evoked from the digital nerves of toes 2 and 4, and recorded with the TIME. The digital nerves were stimulated by means of two small needles at the side of toes second and fourth, delivering pulses of 200 μs at 1 Hz, with increasing amplitude 1–10 V at steps of 1 V. Ten CNAPs obtained for each voltage pulse were averaged to determine the initial latency and the peak amplitude. The CNAP peak corresponding to Aαβ fibers was identified from its conduction velocity, and the mean maximal amplitude plotted as

recruitment curves. A CNAP was considered when its amplitude exceeded two times the maximum value of the root mean square of the background noise. For each active site, a selectivity index (SIas) was calculated as the ratio between the CNAP amplitude exhibited for one stimulated digital nerve, CNAP toe i, and the sum of the CNAP amplitudes elicited in the two digital nerves (Veraart et al., 1993) as in the formula [1].

With the aim of assessing the capabilities of TIME for recording functional neural signals preferentially in different active sites and the possible discrimination of sensory modalities, two different experiments were performed. The first was designed to evaluate the spatial selectivity of afferent signals recorded with the TIME. For this purpose, a pressure stimulus was applied with a Von Frey filament on pad A, toe 2, and tip 2 in the medial side of the paw, and on pad D, toe 4 and tip 4 in the lateral side of the paw. The signal-to-noise ratio (SNR), expressed as the ratio of the signal RMS to the noise RMS, was calculated for each of the six active sites implanted within the tibial fascicle, that innervates the plantar surface of the rat hindpaw. In the second experiment, the objective was to assess the SNR values for stimuli of different sensory modalities. Three tactile, one proprioceptive, and one nociceptive mechanical stimuli were applied in this order: (1) soft tactile stimulus by brushing the sole with a small brush, (2) pressure on a plantar pad, (3) fast scratch on the midline of the hindpaw, (4) flexion of the toes of the paw, and (5) light prick of the plantar skin with a small needle (Raspopovic et al., 2010). Each test was repeated 10 times with intervals of at least 10 s between stimuli.

The recorded neural signals were then bandpass filtered by means of a finite impulse response (FIR) filter with 0.4 and 2.2 KHz cut-off frequencies, preserving the main power of the ENG signal. The mean absolute value (MAV) was extracted from the preprocessed data using a bin width of 100 ms and an overlap of 50% (Raspopovic et al., 2010). Finally, a Support Vector Machine (SVM) classifier was used to discriminate between the different stimuli. Data were divided in a Training set (Tr) consisting in a random selection of 50% of the trials, and in a Testing set (Tt) consisting in the rest of the data. The SVM classifier was trained with Tr using the optimal parameters, and tested with Tt during the discrimination of: (i) one stimulus vs. background activity, (ii) two stimuli and background activity, and (iii) three stimuli and background activity.

6.2.2.2 Results

Different active sites of the TIME selectively recorded the CNAP evoked by stimulating the digital nerves of toe 2 or toe 4, indicating that it was

possible to obtain topographically selective recordings of neural signals from different targets at the subfascicular level with the TIME. The mean onset latency of the measured CNAP peaks was $\sim 2.0\,\mathrm{ms}$, which corresponds to a mean conduction velocity of $\sim 40\,\mathrm{m/s}$, as expected from a population of large myelinated mechanoreceptive fibers (Harper and Lawson, 1985). The stimulation threshold for recordings in AS1 and AS2 of both branches of the TIME was 5–7 V whereas for AS3 it was above 12 V. This fact is related with the position of the electrode inside the nerve trunk. Active sites placed near the target stimulated nerve bundle record the evoked CNAP at lower levels of stimulation than active sites placed at more distance that would require stimulation of more nerve fibers.

From the recruitment plots of the amplitude of the CNAP recorded in the tibial nerve with increasing magnitude of stimulation, we calculated the SIas. In three animals, it was possible to discriminate the CNAP coming from toe 2 and toe 4 with a SI higher than 71%. In other two rats, the SI was 100% for toe 2 but only 55% for toe 4, while in the remaining rat the SI was 73% for toe 2 and 56% for toe 4. The selectivity indices for active sites AS3, which were probably implanted in the region of the tibial nerve occupied by nerve fibers innervating muscles of the hindlimb, were lower than for AS2 and AS1, which were located within the subfascicles of the tibial nerve going to the plantar nerves (see Figure 6.3) (Badia et al., 2016).

During sensory stimulation of the plantar aspect of the hindpaw, we recorded bursts of action potentials from the TIME active sites. Afferent neural activity elicited by tactile, proprioceptive, and nociceptive stimuli did not show significant differences in the SNR level. The mean value of the SNR, being higher with the application of fast scratch stimulation than static pressure or pricking stimulation. For the same type of stimulus applied in one of the stimulation spots, the SNR of the evoked recordings varied in amplitude between different ASs; the SNR was highest at AS1, and lowest at AS3. These results are attributed to the location of AS1 closer to the bundle of sensory fibers that innervate the hindpaw than the other ASs (Figure 6.3). Thus, the TIME may give spatial discrimination of source of the recorded neural activity.

The capacity to discriminate different sensory activity was tested by using an automatized classifier. Considering the mean classification accuracy for the best channel in each of the rats, it was possible to robustly discriminate between different stimuli and background activity from the ENG signals recorded with the TIME. Mean values of 95.4%, 96.0%, and 85.2% were obtained when discriminating pressure, proprioceptive and nociceptive stimuli vs. background, respectively.

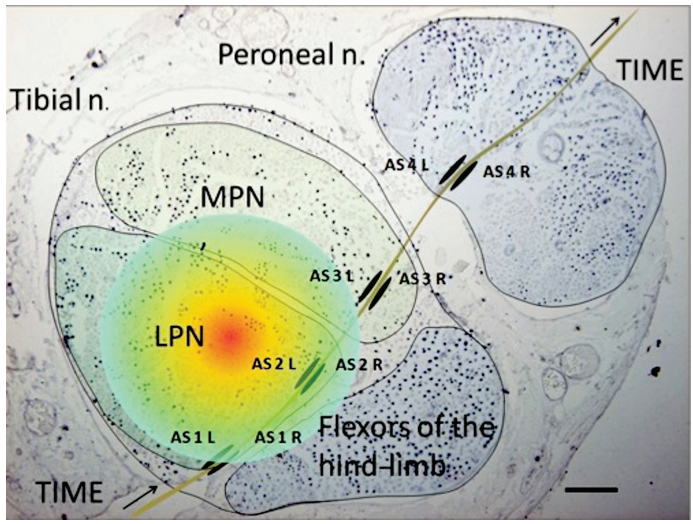

Figure 6.3 Microphotograph of a transverse section of a sciatic nerve immunolabeled against cholin-acetyl transferase (ChAT, dots stained in black) to label motor axons, and counterstained with hematoxilin to visualize the tissue. The narrow strip occupied by the intraneural portion of the TIME has been overlaid with a thin line (brown) and the active sites (AS, in black) marked in a possible position.

6.2.2.3 Discussion

The results of these works made on the rat model indicate that the TIME allows highly selective stimulation of different small muscular fascicles and even parts of the same fascicle within the sciatic nerve of the rat (Badia et al., 2011), and that it also offers good capabilities for selective recording of neural signals elicited by electrical and mechanical stimulation delivered at discrete areas of the foot of the rat (Badia et al., 2016). Furthermore, the post hoc processing of the signals recorded from the TIME allows the identification of the corresponding stimulus pertaining to different functional modalities. Relative comparison of the SI obtained suggests that the TIME may be more selective for stimulation of nerve fibers than for recording neural activity. The neural signal recorded is the superposition of all the afferent signals corrected by the distance factor, whereas in the case of stimulation, since an axon is not activated until the threshold of stimulation is reached, only the nearest axons to the active site will be activated at low levels of current, and therefore spatial selectivity will be present (Raspopovic et al., 2012).

Because of the careful insertion inside nerve fascicles, TIMEs and LIFEs achieved muscle activation at a considerably lower intensity than the extraneural cuff electrodes, further supporting the advantage of intraneural

electrodes for reducing the amount of current needed for axonal stimulation. The intraneural placement of the active sites also allowed meaningful recordings of neural signals evoked by functional stimulation of afferent axons in the rat sciatic nerve. The recordings were usually of integrated action potentials, with seldom spikes, comparable to those obtained with other intrafascicular electrodes (Branner and Normann, 2000; Yoshida et al., 2000; Navarro et al., 2007).

In the comparative study for stimulation, the TIME was the only electrode tested that provided good stimulation selectivity at interfascicular and also at intrafascicular level with one electrode implanted, even in a small size nerve such as the rat sciatic nerve. In contrast, the LIFE allowed only selectivity at the intrafascicular level, and the multipolar cuff electrode only interfascicular selectivity. Thus, the TIME has advantage in the relation between selectivity and invasiveness. Even when the targeted muscle was stimulated above 30% of the maximal CMAP, a functionally relevant magnitude (Bao and Silverstein, 2005; Paternostro-Sluga et al., 2008), the SId of TIME remained higher than those of LIFE and cuff electrodes. These results suggest that the optimal application of TIME device may be in the portions of the peripheral nerve where different bundles of axons innervating different organs are in the inner part of the nerve forming or not a fascicle encircled by perineurium. By repositioning the TIME short distances in the transversal direction, the selectivity of single active sites may be considerably improved, suggesting the possibility of measuring the SIas of the TIME implant and then slightly adjust its position in the nerve to optimize stimulation outcome. Once the optimal position is achieved, the electrode can be fixed in place to the nerve to avoid undesired displacements.

The works here summarized provide evidence that the TIME is a good intraneural electrode to be used as a bidirectional interface between neuroprostheses and the peripheral nerves. The TIME may be appropriate for neuroprosthetic applications at mid and proximal levels of the nerves, reducing the number of electrodes to be surgically implanted and thus minimizing the risk of damage and the complexity of the interface connections.

6.3 Evaluation of TIME in the Pig Nerve Model

The pig nerve model was used for testing the TIME electrodes, because of pig nerves anatomical resemblance to humans nerves in terms of number of fascicles and nerve diameters. Two studies were conducted: (1) Initially acute studies were performed to develop and test implantation techniques and with the purpose to assess selectivity performance and required recruitment current

of the TIME electrode; (2) Chronic studies were later conducted to assess the selectivity performance, recruitment current, stability and biocompatibility of the TIME (see chapter 5 also) during periods of implantation.

6.3.1 Acute Study of Stimulation Selectivity

TIMEs and tfLIFEs were implanted into the median nerve of the left foreleg of farm pigs (Kundu et al., 2014). The TIMEs were implanted transverse through the center of the nerve and the tfLIFEs were implanted parallel to the nerve direction. Patch electrodes were sutured to the seven muscles innervated by the median nerve distally for recording the evoked electromyogram (EMG). Stimulation was performed using monopolar rectangular $100\ \mu s$ duration current pulses, gradually increased in intensity from 40 to $800\,\mu A$, with individual TIME/tfLIFE active sites as cathode. The evoked EMG responses from the monitored muscle were used as an indirect assessment of the nerve recruitment evoked by the tested TIME and tfLIFE electrodes. The EMG recruitment level in percentage ($\mathrm{EMG_{RL}}$) for each muscle, was calculated by taking the normalized root mean square value of EMG response. The selectivity for each muscle ($\mathrm{SI_m}$), was then calculated using the same selectivity index as in the rat experiments described above. As a constrain to reduce the influence of noise in the EMG recordings, $\mathrm{SI_m}$ was calculated only when the target muscle was activated $> 30\%$. The specific 30% limit was defined as a minimum requirement for the recruitment to be functionally relevant (Bao and Silverstein, 2005). To evaluate the performance of a whole TIME/tlLIFE electrode a device selectivity index ($\mathrm{SI_d}$) was defined as the average of the maximal $\mathrm{SI_m}$ achieved for each of the seven monitored muscles. Finally, the required recruitment current was calculated as the average current used, when achieving the maximal $\mathrm{SI_m}$.

After ending the stimulation protocol, the pig was euthanized. For eight TIMEs and four tfLIFEs the location of the electrode inside the nerve was determined via histology. For these instances the nerve was carefully dissected free and harvested after which it was frozen in liquid nitrogen, sectioned into $5\ \mu m$ cross-sections and stained with Hematoxylin and Eosin.

6.3.1.1 Results

Results showed that individual muscles could be most selectively activated at the lower current levels. When using higher currents, the selectivity dropped, as more parts of the nerve were recruited. The TIME was on average capable of recruiting 2.2 ± 0.9 muscles to a $\mathrm{SI_m} > 0.5$ whereas the tfLIFE on average only selectivly activated 1 ± 0.0 muscles. Overall the TIME had a higher $\mathrm{SI_d}$

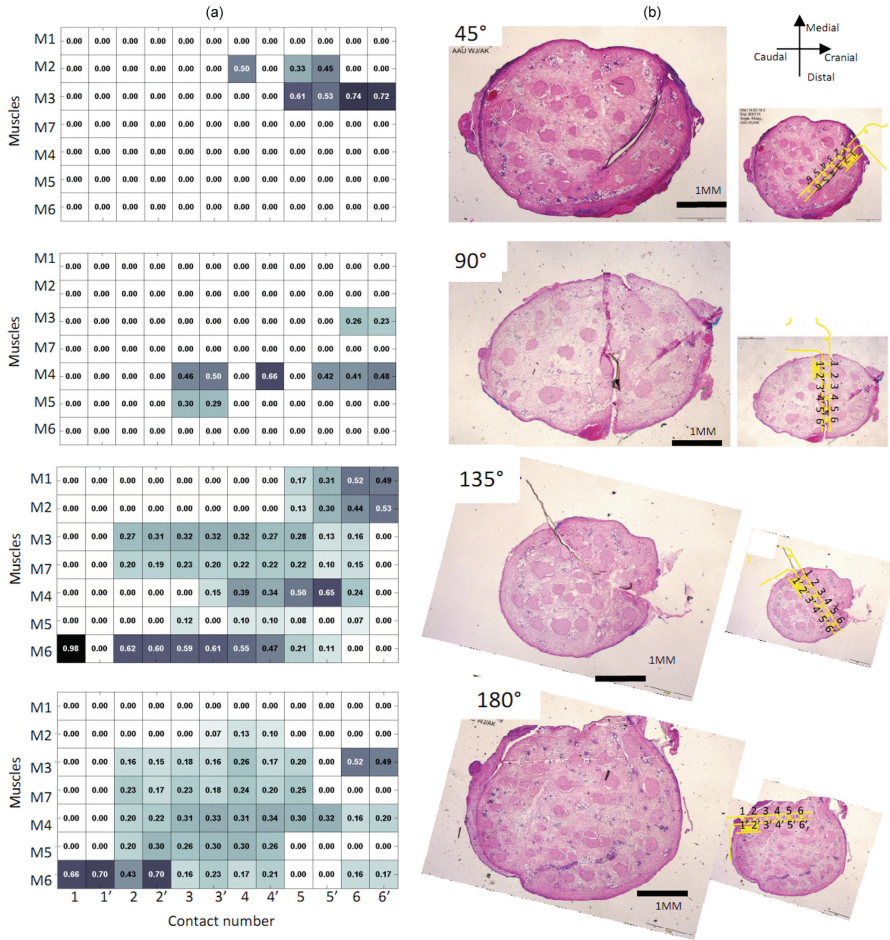

Figure 6.4 Heat maps indicate the selectivity achieved for individual muscles (M1–M7), 0 corresponding to white and black corresponding to 1, when using the different contact sites of the TIME (1–6 and 1'–6', corresponding to the contact sites on each side of the TIME loop structure) and tfLIFE (1,1–4,4'). The histology images show the corresponding traces of the inserted electrodes. In general, the TIMEs were better at activating several different muscles, whereas the tfLIFE tended to activate a single muscle selectively. Reprinted with permission from Kundu et al. 2014.

than the tfLIFE ($p = 0.02$) and performance was not found to be related to the angle with which the electrodes had been implanted into the nerve (see Figure 6.4). With respect to the required current, I_d, no differences were found between the two type of electrodes. Histological assessment, showed that all

the electrodes had been placed between fascicles, and not inside the fascicles as intended.

6.3.2 Chronic Study of Stimulation Selectivity

Four female Göttingen mini-pigs (25–40 kg) were implanted with a total of six TIMEs over a period of >30 days (Harreby et al., 2015). TIMEs were always implanted through the center of the nerve; when implanting two electrodes in the same nerve, they were inserted at different angles to cover different subsections of the nerve. TIMEs were sutured to the epineurium using the anchoring points just at the entry point and at the ribbon, which connected it to the ceramic connector, see Figure 6.5A. The ceramic connector was fixed to tissue close to the nerve.

EMG patch electrodes were sutured onto five muscles innervated by the median nerve and a cuff electrode was attached to the median nerve to provide supramaximal stimulation.

The ceramic connector of the TIME implant was connected to a 16 channel circular connector (Omnetics Inc., Minneapolis, USA) via helically coiled MP35N wires enclosed in silicone tube shielding. The lead out wires from the EMG and cuff electrodes were also connected to a 16 channel circular connector. The silicone tube cables from TIMEs, cuff and EMG electrodes were tunneled subcutaneously to the back of the pig and mounted in a custom made stainless steel capsule, which was sutured to the skin.

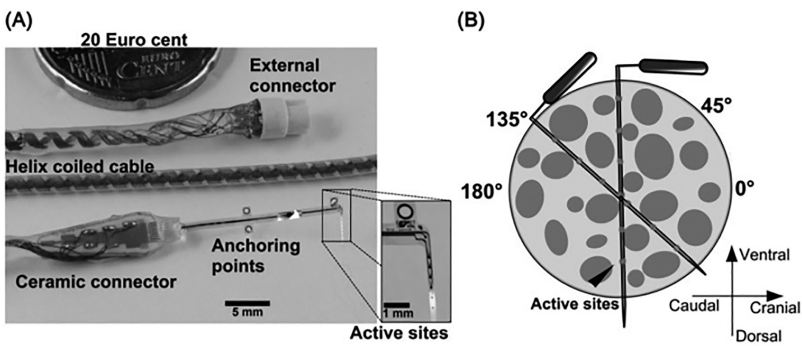

Figure 6.5 (A) picture of the TIME implant. (B) Illustration indicating the TIMEs were located inside the nerve. (B) Example was TIME electrodes are placed at 135° and 90°. As in the acute pig study, post-mortem findings showed the TIMEs had been located between the fascicles. Reprinted with permission from Harreby et al. 2014.

6.3.2.1 Follow-up methods

Follow-up sessions were performed two to three times a week for the duration of the implantation period. During these events stimulation and recording equipment was connected to the lead out wires via the Omnetics connectors at the back of the pig.

All stimulation was conducted at 2 Hz using rectangular $100\ \mu\mathrm{s}$ current pulses. Stimulation current gradually increasing up to $1200\ \mu\mathrm{A}$, corresponding to the maximal charge capacity of the TIME (Boretius et al., 2012). A total of 22 stimulation configurations were evaluated for each TIME electrode: 12 corresponding to monopolar stimulation using each contact site (1–6 and 1'–6'). Then 10 nearest neighbor bipolar configurations were used (1–2 ... 5–6 and 1'–2' ... 5'–6').

In some instances EMG channels became unstable, in these cases monopolar EMG recordings were made when possible. If monopolar recording could not be satisfactorily obtained, the specific channel was omitted from the analysis.

During offline analysis three different techniques were applied to clean the EMG recordings for noise: (1) Stimulation artifacts were blanked out and replaced by a linear interpolation, (2) the signal was band pass filtered between 0.1 and 2 kHz, and (3) power line noise was reduced by subtracting a "quiet" but noisy recorded signal segment 20 ms (period of powerline noise) prior to the segment of interest. Evoked EMG activity was now quantified using the RMS value as in the acute rat and pig studies.

The required current for activating a particular muscle, ($I_{m30\%}$) was determined as the minimal current for any TIME contact configuration to obtain a recruitment level of 30% ($EMG_{RL30\%}$). The minimal required current for recruiting all monitored muscles using a single TIME device ($I_{d30\%}$), was simply calculated as the average $I_{m30\%}$ for each of the individual muscles monitored.

The selectivity index used in this study was based on the one used in the acute rat and pig study, but with a couple of adaptations/improvements. Initially the constant weighting SI ($\mathrm{SI_{CW}}$) was defined as:

$$\mathrm{SI}(I)_{\mathrm{CW,j}} = \frac{\mathrm{EMG}(I)_{\mathrm{RL,j}}}{\mathrm{EMG}(I)_{\mathrm{RL,j}} + (N_C - 1) * \left(\frac{\sum_{i=1|\mathrm{i}\neq j}^{N_A} \mathrm{EMG}(I)_{\mathrm{RL,i}}}{N_A - 1} \right)}$$

Here N_C, is a constant corresponding to the number of muscles intended to be monitored, and N_A is the number of muscles from which EMG could

actually be recorded. This reduces the bias induced if EMG could not be recorded from all muscles, and thus the number of EMG channels changed. If $N_C = N_A$, $\mathrm{SI_{CW}}$ simply corresponds to the selectivity indices used in [1] and [2].

To ensure $\mathrm{SI_m} = 0$ when all muscles were equally activated, the following correction was applied to $\mathrm{SI_{CW}}$:

$$\mathrm{SI(I)_{m,j}} = \frac{N_C}{N_C - 1} * \left(\mathrm{SI(I)_{CW,j}} - \frac{1}{N_C} \right)$$
$$\text{if} \quad \mathrm{SI_{m,j}} < 0 \quad \text{then} \quad \mathrm{SI(I)_{M,j}} = 0$$

This function can be calculated for each contact combination. $\mathrm{SI_{m\geq 30\%,j}}$ was now defined as the highest $\mathrm{SI_m}$ value which could be achieved by a single TIME contact combination, when the recruitment level was $\mathrm{EMG_{RL30\%}}$ or above. A muscle was defined as being selective activated when $\mathrm{SI_m} > 0.4$, corresponding to an average nontarget recruitment of 1/4 of that of the target muscle. To quantify the overall selectivity performance of a whole TIME device the $\mathrm{SI_{d>30\%}}$ was defined as the mean of all $\mathrm{SI_{m\geq 30\%}}$ values.

6.3.3 Results

Six TIMEs were implanted in four pigs for a total of 33.8 ± 2.4 days. Current pulses evoked EMG responses, occurring mainly between 3 and 10 ms after the stimulation pulse onset, see Figure 6.6. Recruitment of muscles in general started at the same current level, but individual muscles often differed in how they were recruited at higher currents, these differences meant that some muscles became more selectively recruited than others. Recruitment curves were in general not smooth sigmoid curves, but rather increased with a mix of increases and plateaus (see Figure 6.7b, c). In general monopolar stimulation evoked more muscle activity than bipolar stimulation did at similar current levels. During the first follow-up around half of the bipolar configurations were able to evoke $\mathrm{EMG_{RL}} > 30\%$, however, during the final follow-up session only 3/60 could evoke such EMG. Initially, in five out of six TIMEs all channels could evoke at least one EMG channel to $\mathrm{EMG_{RL}} > 30\%$. Two TIME electrodes gradually stopped being able to recruit muscles, however, the remaining electrodes could recruit to $\mathrm{EMG_{RL}} > 30\%$ on nearly all contact sites until last follow-up. The average $I_{d30\%}$ was initially 488 ± 68 μA and increased to 769 ± 128 μA during the last follow-up.

The $\mathrm{SI_{d\geq 30\%}}$ started at 0.25 ± 0.04 during the initial follow-up and gradually decreased to 0.14 ± 0.05 during the last follow-up ($P = 0.12$).

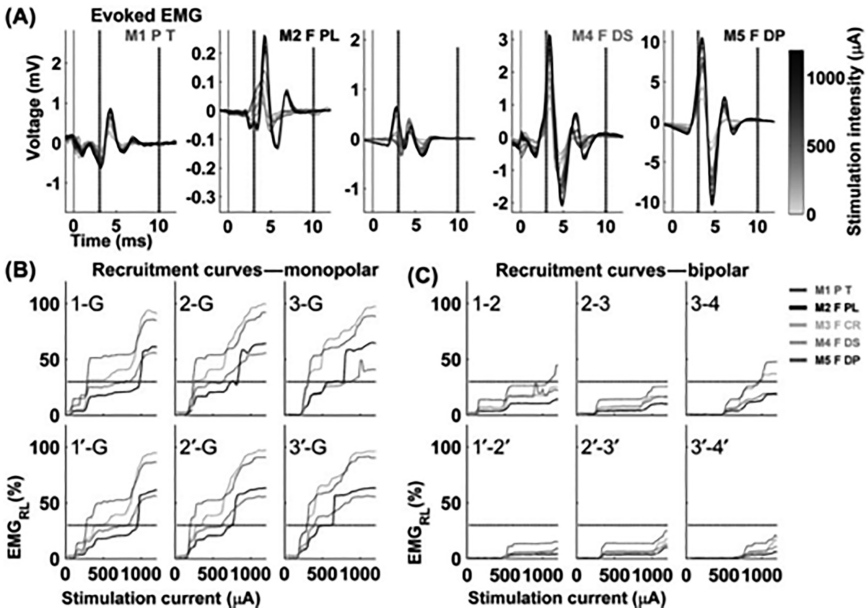

Figure 6.6 (a) The raw evoked EMG response when stimulating in P2T1 at day 7 from the five monitored muscles when stimulating monopolar using 3'. The dotted vertical lines indicate 30% EMG recruitment ($\mathrm{EMG}_{\mathrm{RL30\%}}$). (b) Shows the recruitment $\mathrm{EMG}_{\mathrm{RL}}$ for each of muscles during monopolar stimulation (G–Ground) with a subset of six contact sites of P2T1. Note that the recruitment curves are not smooth, but rather have consist of steep increases and plateaus. (c) Shows the recruitment curves related to a subset of bipolar stimulation configurations. Note that the recruitment level is significant lower for bipolar stimulation than for monopolar stimulation. Reprinted with permission from Harreby et al. 2014.

Based on our definition of selectively activated muscles, each TIME could initially activate 1.17 ± 0.37 muscles, which dropped to 0.67 ± 0.38 during the last follow-up ($P = 0.18$). Neighboring contact sites on the TIME (same side and on opposite sides), tended to recruit the same muscles, with only small variations in the recruitment curves. Electrodes P2T1 and P2T2 which were both implanted in the same nerve, recruited different subsets of muscles (Figure 6.7a, b). During the initial follow-up bipolar stimulation configurations were selected at the best stimulation configuration in 1/3 of cases, when calculating the $\mathrm{SI}_{\mathrm{d}\geq 30\%}$, however, if these configurations were left out of the overall $\mathrm{SI}_{\mathrm{d}\geq 30\%}$ calculation (i.e., based only on monopolar stimulation) the value dropped only by 0.005, indicating that bipolar configurations contributed insignificantly to the selectivity.

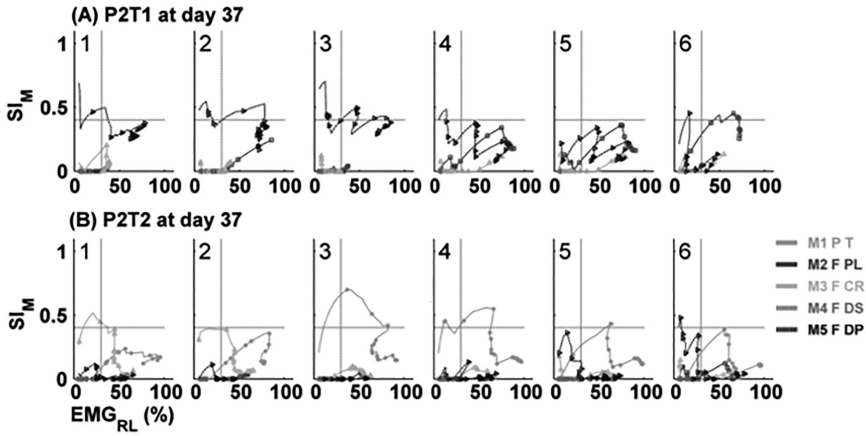

Figure 6.7 The selectivity of individual muscles is shown as a function of muscle recruitment level for a subset of monopolar stimulation configurations from electrodes P2T1 and P2T2 during the last follow-up session at day 37. The vertical and horizontal dotted lines indicate the limits for $\mathrm{EMG}_{\mathrm{RL30\%}}$ and $\mathrm{SI}_{\mathrm{m}} = 0.4$, thus based on our definitions a muscle is selectively recruited if it enters the upper right quadrant. In P2T1 muscles: M5 and M2 are selectively activated, in P2T2 M3 and M1 are selectively recruited. Reprinted with permission from Harreby et al. 2014.

6.3.4 Discussion

The acute pig experiments showed that the TIME design was more effective than the tfLIFE to selectively activate different parts of a large polyfascicular nerve. This is not surprising as the tfLIFE was designed to be placed in parallel to the nerve, meaning that all the contact sites of one tfLIFE would be placed close to the same few fascicles in the nerve. In contrast, the TIME was implanted transversal through the nerve, which means that the contact sites were placed along the whole cross-section of the nerve.

Although different methodologies were used and thus it is difficult to compare directly, the selectivity seen in the pig model seem to be lower as those achieved by implanting TIMEs in rats (Badia et al., 2011). There may be several reasons for this. Badia et al. were able to insert the TIMEs directly into the target fascicles of the small rat nerve, this resulted in a much smaller recruitment current and a better selectivity. In the large nerve of the pig it was not possible for us to insert into specific fascicles. Furthermore, the location of the specific fascicles which innervated the muscles from which EMG were recorded was not known. This meant that electrodes were inserted “blindly”

through the epineurium. Inside the nerve, the needle then glided of the tough perineurium of individual fascicles which resulted in the extrafascicular placement. This location both increased the required recruitment current needed and reduced the achievable selectivity. Methods to overcome the extrafascicular location in the future could be to make previous neurolysis of the nerve to separate the fascicles or to insert the guide needle at high velocity, as have been done when placing the Utah array (Warwick et al., 2003).

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