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REGISTRATION OF SOMATOSENSORY EVOKED POTENTIALS IN THE LUMBAR AND LOWER THORACIC SPINE USING HIGH-DENSITY SURFACE ELECTROMYOGRAPHY

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Samenvatting

Het beoordelen van activiteit van het ruggenmerg wordt gedaan met behulp van ElectroSpinography (naald EMG). Dit is een pijnlijke en invasieve methode waarbij naaldelectrodes in de subarachnoïdale ruimte worden gestoken. Er wordt verwacht dat High-Density oppervlakte elektromyografie (HD-sEMG) een non-invasief alternatief kan zijn voor naald EMG. Het doel van dit onderzoek is om te bepalen of HD-sEMG toegepast kan worden om neurofysiologische activiteit in het ruggenmerg te monitoren.

Vijf proefpersonen hebben elektrostimulatie van de n. tibialis posterior ondergaan. Drie configuraties van de HD-sEMG grid (64-kanaals) zijn gemeten tijdens deze stimulaties; (1) Net lateraal van de L4 wervel, (2) Centraal op de processus spinosis van L4, (3) Centraal op de processus spinosis van Th12. Iedere configuratie is twee keer gemeten per proefpersoon. Het stimulatieprotocol bestond uit duizend pseudo-willekeurig toegediende (±3 Hz) stimulaties. Het HD-sEMG-signaal werd gesampled met 16348 Hz.

Het gemiddelde van de duizend responses op de stimulaties is bepaald voor alle kanalen van elke meting. Een tijdsinterval van 0.01 tot 0.30s is gebruikt. Over duizend keer datzelfde tijdsinterval is ook het gemiddelde signaal zonder stimulaties bepaald, de baseline response.

Alle gemiddelde stimulatieresponses zijn beoordeeld voor duidelijk zichtbare pieken, Somatosensory evoked potentials (SSEPs). Het gemiddelde vermogen van deze SSEPs is gedeeld door de standaardfout van de baseline response om de signaal-ruis verhouding (SNR) te bepalen.

De SNRs van configuratie 1, 2 en 3 waren 5.23 (±3.56), 3.96 (±1.23) en 3.96 (±2.03) dB, respectievelijk. Deze verhoudingen suggereren dat HD-sEMG toegepast kan worden om de neurofysiologische activiteit in het ruggenmerg te monitoren.

Contents

Abstract

The current procedure for assessing nervous activity in the spinal cord is needle ElectroSpinography (needle EMG). This is an invasive and painful approach during which a needle is inserted into the subarachnoid space. It is hypothesised that High-Density surface ElectroMyography (HD-sEMG) is a non-invasive alternative for this. This study was conducted to test the viability of HD-sEMG for monitoring spinal functioning.

Five subjects underwent electro-stimulation of the n. tibialis posterior. Three configurations for a HD-sEMG grid (64 channels) were tested: (1) just lateral of the L4 vertebra (towards side of stimulated limb), (2) on the processus spinosis of L4, and (3) on the processus spinosis of Th12. Each configuration was recorded twice per subject. The stimulation protocol consisted of 1000 stimulations administered pseudo-randomly every ±300 ms, while the HD-sEMG was sampled at 16 kHz.

Epoch 10-300 ms after every stimulation were aligned and averaged. This yielded a single average response per channel, which was compared against a baseline (recording without stimulations).

All channels were assessed to identify significant, stimulus-related potentials. The average power of the peaks was divided by the standard error of mean of the baseline to obtain the signal-to-noise ratio (SNR).

The SNRs of configurations 1, 2, and 3 were 5.23 (±3.56), 3.96 (±1.23), and 3.96 (±2.03) dB, respectively. All three SNRs were found to be sufficiently high to suggest that HD-sEMG is indeed a viable technique for monitoring neurophysiological activity in the spinal cord.

Introduction

Diagnostics of neuropathies of the spinal cord

The spinal cord is of paramount importance for peripheral motor and sensory functioning in humans. How can its anomalous functioning be identified? Answering this question may be of great relevance for diagnosing motor and sensory impairments. However, the current procedures for assessing the spinal functioning come with certain shortcomings.

The most common manner of assessing the functioning of the spinal cord is done indirectly, through physical examination of a patients symptoms. Localized numbness, (partial) paralysis, and pain can be readily assessed. Given a thorough knowledge about neuro-anatomy one may infer underlying spinal impairments from this. Unfortunately, for neurological impairments, this procedure provides little to no information on the precise anatomical level or location of the impairment along the neural pathway. Moreover, not all symptoms can be pinpointed back to a single direct cause this way. Consequently, it is often necessary to explore the symptoms further using more advanced techniques to determine whether its origin is anatomical or neurological.

Magnetic Resonance Imaging (MRI) is a valuable addition when it comes to examining the spinal cord. MRI is used to reveal abnormal structures. In the soft tissues around the spinal cord it can expose: abscesses, hematomas, tumours, and ruptured discs. It also serves to detect anomalies in the bone surrounding the spinal cord, exposing: cervical spondylosis, fractures, and tumours. Information provided by MRI can also differentiate grey matter from white matter. However, a spinal MRI-scan has its limitations. It shows the white matter of the spinal cord as a uniform tissue, whereas it in reality is an organized structure of directionally oriented nerve fibres (Fujiyoshi, et al., 2007). Also, registering the neurophysiological activity in the spinal cord is out of the scope of MRI. Although brain activity can be recorded using *functional MRI* (fMRI), the absence of blood flow to individual fibres in the spinal cord prevents this for spinal activity. Furthermore, a MRI machine is expensive and implanted devices (pacemakers) or metal limit its usefulness.

An alternative technique is myeolography; this is a *Computed Tomography* (CT) scan with a contrast agent. While being less costly than MRI, it is also less detailed and unable to detect spinal activity. For the assessment of the spinal cord, it is required to inject the contrast agent in the subarachnoid space.

MRI and myeolography are both techniques used to reveal anatomical anomalies that influence spinal function. To identify functional anomalies in spinal functioning the neural

activity needs to be registered. The technique that is most commonly used for this purpose, is needle *ElectroSpinography*, which in terms of measurement equals the conventional needle *ElectroMyography* (EMG). This is an invasive procedure where needles are inserted in the subarachnoid space to detect the electric potentials in the spinal cord (Desmedt & Cheron, 1980). It provides a highly detailed, albeit spatially very constrained, measurement of spinal activity.

Somatosensory Evoked Potentials

Needle EMG allows for measuring electric potentials in the spinal cord. However, the spinal cord consists of a vast number of nerve fibres that each pass along different afferent or efferent information simultaneously. The resulting signal consists of a non-trivial composition of different potentials. This signal cannot be distinguished or interpreted, as the origin of each contributing source is unknown. As a result, the sole registration of this mix of potentials, without knowledge of its origin, yields insufficient information to confirm a diagnosis. To confirm a diagnosis, one must know the spinal activity and how it deviates from the healthy activity. To gain this knowledge it is common practice to use a standardized method where a peripheral nerve is stimulated via an electro-stimulation protocol. The neural response to each stimulation, a *Somatosensory Evoked Potential* (SSEP), travels through the nerve and via the spinal cord to the brain. These SSEPs can be registered in the spinal cord using needle EMG (Nuwer, 1998). The measured SSEPs, their known moments of origin, and the expected response a healthy nerve would show, are combined to form a more complete picture of the functioning of the spinal cord. The SSEPs are very susceptible to impairment. As a result, this method can be used for several clinical purposes (Chawla, Burneo, & Barkley, 2016):

1. To gather quantitative evidence of abnormality.
2. To test for lesions before they manifest themselves clinically.
3. To detect the anatomical location of a lesion along a potentials' pathway.
4. To provide evidence about the general category of the pathology.
5. To objectively monitor the change in spinal activity over time.
6. To safeguard the (central) nervous system during (high-risk) surgery.

Needle EMG is the conventional procedure for measuring the SSEPs. Next to the aforementioned limitation to very focal sources, the main shortcoming with this test is its invasiveness. A needle to the subarachnoid space of the spinal cord can be particularly painful and is not free of risk. The spinal cord is highly sensitive and damage to it can have far reaching consequences. An alternative, non-invasive, risk free technique to register the SSEPs in the spinal cord is therefore desired.

HD-sEMG as alternative procedure

Originally, the purpose of needle EMG is to measure muscle activity. For this goal, surface EMG (sEMG) is its non-invasive alternative. This raises the question whether sEMG is also a viable alternative for needle EMG when it comes to measuring spinal activity.

The common bipolar sEMG configuration proved to be insufficiently equipped to provide accurate information on muscle and neural activity for diagnostic purposes (Linsen, et al., 1991). However, High-Density sEMG (HD-sEMG) is believed to be adequately equipped to register the spinal activity. At the VU department of Human Movement Sciences, an initial pilot study has been conducted to detect neural activity of the spinal cord after peripheral electro-stimulation (Luger & Daffertshofer, 2016). Using HD-sEMG grids above lumbar vertebrae L4 and L5 reliable activity patterns have been found with a high signal-to-noise ratio (SNR) after 1,000 stimuli. However, the spatial coverage during this pilot study was about 8 cm and the analog-to-digital convertor (ADC) involved was limited to a sampling rate of 2048 Hz. Although the activity pattern was found to be reliable with a high SNR, it remains to be confirmed that the signal recorded during this pilot was indeed neural activity in the spine, not neural activity from the plexus lumbalis or dorsal root of the sciatic nerve. If this is the case, it may confirm HD-sEMG as a reliable and non-invasive test for measuring SSEPs.

The aim of this study is to determine whether HD-sEMG is indeed adequately equipped to register SSEPs in the spinal cord. The neural activity is examined on three locations on the lower back and state-of-the-art HD-sEMG

equipment is used with a sampling rate of 16.384 Hz and a stimulation protocol of 1.000 stimuli to the tibial nerve.

Method

Subjects

Five young (mean 23.2 yrs, SD 1.3) and healthy adult subjects (3 male/2 female) participated in this study.

Preparation

Prior to conducting the experiment, the subject was informed of, and prepared for, the procedure. Prior to the application of the electrodes, the skin of the subject was shaven and cleansed with alcohol. This minimalized the impedance between the skin and the electrode, which decreases the noise in the measured signal and increases the statistical power (Kappenman & Luck, 2010). Subsequently, the electrodes for the electro-stimulation system (Dura Stick pads) were placed. The cathode for the stimulation was placed between the medial border of the Achilles tendon and the posterior border of the medial malleolus. The anode was located 3 cm in distal direction – see Figure 1. A ground electrode was placed on the caput fibula of the same limb to reduce stimulus artefact – see Figure 2.

To measure the neural response, three configurations for the placement of the 64-channel HD-sEMG grid (ANT-neuro eego^{tm}-mylab, 8x8 electrodes, 4mm inter-electrode distance) were used – see Figure 3.

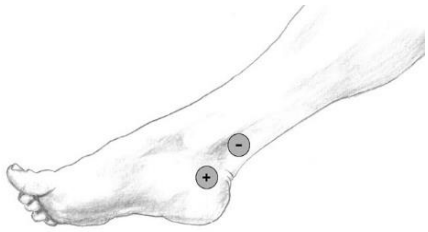

Figure 1: Placement of the anode (+) and cathode (-) electrodes for electro-stimulation of the tibial nerve.

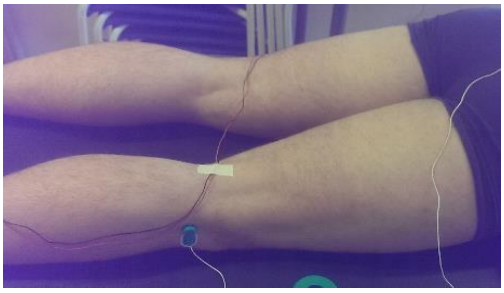

Figure 2: Placement of the ground electrode (blue) on the caput fibula of the stimulated limb.

1. Lateral of the processus spinosis of the L4 vertebra to the stimulated side. The median edge of the electrode grid touching the midline of the back. This configuration aimed to record the neural activity of the sensory (dorsal) root.
2. Over the L4 processus spinosis. This configuration aimed to record the neural activity of the cauda equina, inside the spine.
3. Over the Th12 processus spinosis. The most distal part of the spinal cord, conus medullaris, is located at the level of L1, L2. Hence, this configuration aims to record the neural activity of the actual spinal cord.

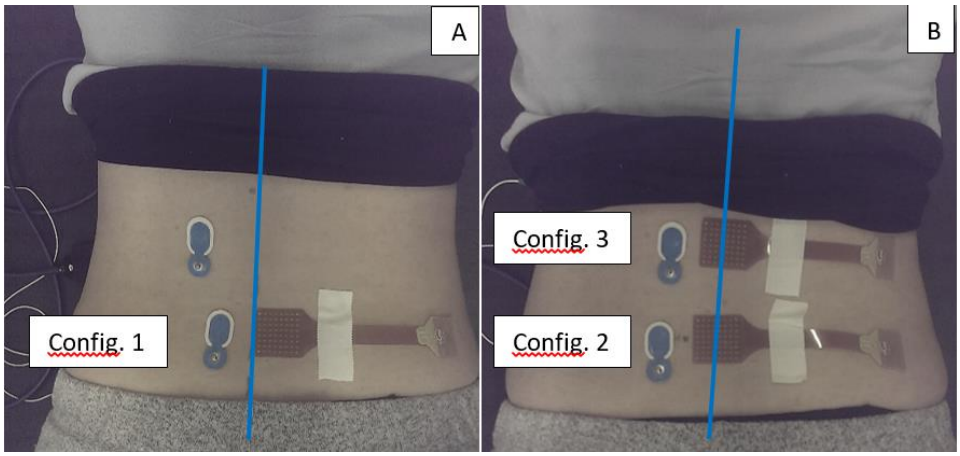

Figure 3: The locations of placement of the HD-sEMG electrode grids. The blue line represents the midline of the subjects' back. The blue electrodes are the reference electrode. The brown square contains the HD-sEMG electrode grid. (A) Config.1: Lateral of L4, in the direction of the stimulated side. (B) Config.2: Centrally on top of the L4 processus spinosis. Config.3: Centrally on top of the Th12 processus spinosis.

Each of these three configurations was accompanied by a reference electrode located 2cm lateral of the electrode grid in the direction of the non-stimulation side.

Stimulation

The subjects lay in a prone position during the course of the experiment. The stimulation protocol was executed in accordance with the AEEGS guidelines (American EEG Society, 1994). As responses to posterior tibial nerve stimulation are subject to less intersubject variability than those to common peroneal nerve stimulation, the peripheral n. tibialis posterior was transcutaneously stimulated (Pelosi, Cracco, Cracco, & Hassan, 1998). Monophasic rectangular pulses with a 0.2 μs duration were delivered at a rate of ±3 Hz. This is within the recommended range of 3-6 Hz but is not a subharmonic of the powerline frequency (50 Hz). Subharmonics of the 50 Hz frequency would cause the 50 Hz noise to be in phase in the intervals following the stimulations, which could possibly lead to contamination with artefacts of the 50 Hz frequency (Legatt, 2014). The stimulation protocol consisted of 1000 stimuli, resulting in a stimulation protocol of 5 minutes and 34 seconds. A pseudo-randomised protocol was used to prevent habituation. The protocol is applied twice for each HD-sEMG grid configuration. The stimulus intensity was kept adequately high to produce a consistent, but tolerable, muscle twitch in the foot or toes of the subject. This was around the intensity of 30-45 mA, since a constant voltage stimulator was used.

Recording

Contact impedance of the surface electrodes was kept as low as possible. The HD-sEMG amplifier recorded the 64 channels of the HD-sEMG grid, the reference and ground electrode, and the trigger from the stimulator. Its sampling rate was set at 16.384 Hz (2^{14} Hz). Each configuration of the HD-sEMG electrodes was measured in a separate trial. The recording was started approximately 10 seconds before the stimulation protocol and ended 10 seconds after it, to also include a measurement of the baseline potential. This resulted in six measurements of approximately 5 minutes and 50 seconds per subject.

Data processing

The recorded signal was offline high-pass filtered with a cut-off frequency of 10 Hz (2^{nd} order bi-directional Butterworth) to correct for the offset and unwanted low frequency noise. The powerline frequency and its first eleven harmonics were removed from the signal using a band-stop filter (2^{nd} order bi-directional Butterworth, bandwidth of 0.2 Hz).

The trigger from the stimulator served to determine epochs in the recorded signal. This resulted in 1000 epochs per recording. The time interval in which the neural response to the electro-stimulation should pass the HD-sEMG electrodes on the lower back was set at 10 to 300 ms after the stimulation. To reduce the noise in the signal, the independent epochs' responses were converged into a single SSEP per channel. This was realized by averaging the time interval following each epoch.

Filtering the noise by averaging independent epochs into a single SSEP relies on the central limit theorem. The central limit theorem states that, when adding independent random variables, their sum tends toward a Gaussian distribution. This is regardless of whether the independent variables themselves are normally distributed. Since ample potentials, or rather ample sources of noise, are measured simultaneously, the distribution of the noise can be assumed to be normal. The mean of the noise is assumed to be (close to) 0 due to the characteristics of measuring potentials with EMG and filtering out the offset with a high-pass filter. This means that, when superimposing the normally distributed noise with a mean of (almost) 0 for 1000 epochs, the result will be close to 0 as well. By contrast, the neural response is not an independent random variable. When summing up the neural responses to the stimulation for 1000 epochs, the result will be 1000 times the neural response. Dividing the summation of the epochs by 1000, causes the resulting SSEP to be of the same size as it originally was but the noise to be greatly diminished.

At this point, all the HD-sEMG channels were inspected for proper functioning during recording and the erroneous channels were removed.

The signal of each channel represents the electric potential between the corresponding electrode and the reference electrode that was located 2 cm lateral of the HD-sEMG electrode grid. To increase the stability and reproducibility of the responses, each channel has been re-referenced to represent the electric potential between itself and the average of the whole grid. This was realized by averaging all the good channels' signals for the whole trial and subtracting this average from the individual channels. The re-referencing to the mean consequently largely removed artefacts from the signal that were present at each channel, such as the ECG artefact.

To provide a compatible parameter for comparison with the SSEPs, the baseline signal was determined. This was accomplished by creating thousand 290 ms (10-300 ms post stimulus) time intervals from the re-referenced signal from the parts before the start and after the ending of the stimulation protocol. The first and last 2 s from the signal were also discarded prior to creating the 1000 baseline intervals, since this part of the signal was distorted. These intervals were again averaged, cancelling out most of the noise, leaving a baseline signal to compare the SSEPs to.

Data analysis

The SSEPs and their corresponding *standard error of mean* (SEM) are plotted in figures, showing the time on the x-axis and the electric potential on the y-axis. The mean of the baseline signal and the *standard deviation* (SD) from the baseline mean are plotted as horizontal lines in the same plot. The stimulus-related responses of every channel were then assessed for clear peaks that surpass the SD of the baseline. To simplify this task, a low-pass filter (1st order Chebyshev) with a cut-off frequency of 1000 Hz was applied to the SSEPs. The sole function of this was to detect possible peaks more readily. When a clear peak was observed, it was used to calculate the (SNR).

The sought-for signal was, in this study, defined as the part of the observed signal that formed a clear peak and exceeded ±SD of the baseline apart from its mean. The noise was defined as the potentials that were present in the signal when no stimulus-related potentials are expected. The stimulus-related potential and the noise were converted into the SNR using

$$SNR = \frac{P_{signal}}{P_{noise}} \qquad (1)$$

where P_{signal} is the power of the stimulus-related potential and P_{noise} is the power of the noise.

Both the stimulus-related potential and the noise were expressed in (micro) Volts, which is a measure of amplitudes. To adjust for this and to make the measure proportional to power the *root mean square* (RMS) amplitude of the signals are calculated (2) and squared, resulting in

$$A_{rms} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(s_i{}^2)} \qquad (2)$$

where the set $\{s_1, s_2, \dots, s_n\}$ represents the signal, and

$$\mathrm{SNR} = \left(\frac{A_{rms.signal}^2}{A_{rms.noise}^2}\right) \qquad (3)$$

where $A_{rms,signal}$ is the RMS amplitude of the signal and $A_{rms,noise}$ the RMS amplitude of the SEM of the baseline.

Following convention the SNR was expressed on a logarithmic *decibel* (dB) scale. Using the definition of decibel, the SNR was hence converted via

$$\mathrm{SNR_{dB}} = 10\log_{10}\mathrm{SNR} \qquad (4)$$

with SNR_{dB}, naturally, being the SNR expressed in dB.

By combining equations (3) and (4), the used formula for calculating the SNR in dB is formed

$$\mathrm{SNR_{dB}} = 10\log_{10}\left(\frac{A_{\mathrm{rms,signal}}^2}{A_{\mathrm{rms,noise}}^2}\right) = 10\log_{10}\left(\frac{A_{\mathrm{rms,signal}}}{A_{\mathrm{rms,noise}}}\right)^2 = 20\log_{10}\frac{A_{\mathrm{rms,signal}}}{A_{\mathrm{rms,noise}}} \qquad (5)$$

Although the time interval of the desired signal and the noise are not equal to one another, this is resolved using the squared RMS of the amplitude. After all, the squared RMS amplitude of an electric signal is proportional to

the average power transmitted by aforementioned signal. The (electric) power, naturally, being the rate (electric) energy is transferred per time unit. Thus rendering the stretch of the time intervals obsolete.

Results

A total of thirty trials were obtained between five subjects. Each trial consisted of 64 channels (Figure 4) that were simultaneously recorded. The total duration of the trials amounts to 175 minutes, resulting in over one week and 18 hours of 16348 Hz sampled data.

Figure 4: Orientation of the matrix of the different electrodes in the HD-sEMG electrode grid. The number located at each electrode site is the number of the channel the electrode corresponds to.

The unprocessed data displayed a high level of noise – see Figure 5. The signal started off at 0 V but increased rapidly to values exceeding $5.5 * 10^4$ µV. When inspecting the data more closely it became evident that this was predominantly caused by the stimulation artefact, as well as the surface potential of the stimulation – See figure 6. Neural responses to stimuli, however, could not be identified. The application of the high-pass filter removed the rapid increase in the first 5 seconds and ensures the absence of an offset.

It was clear from these responses that not all channels functioned properly. Most channels registered responses that regularly oscillated within the bounds of one baseline SD from the mean, with episodic peaks resembling the somatosensory evoked potentials. Some channels, however, presented with a signal that deviated enormously from this expected pattern – see Figures 7 and 8. Such channels were discarded. Many of the channels from recordings of subject 4 exhibited this erroneous signal. To prevent interference from these recordings, subject 4 was discarded. The

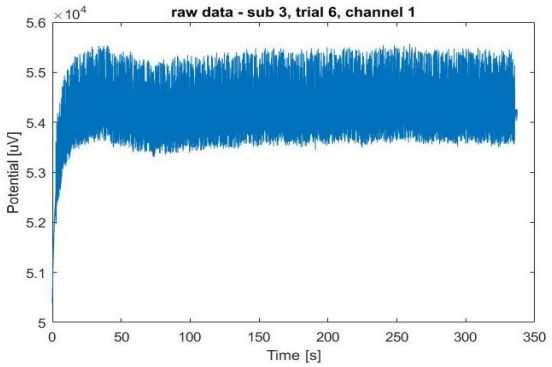

Figure 5: Measurement of neurophysiological activity recorded on the skin surface lateral of L4 vertebra during 1000 stimulations of the n. tibialis posterior, prior to data processing.

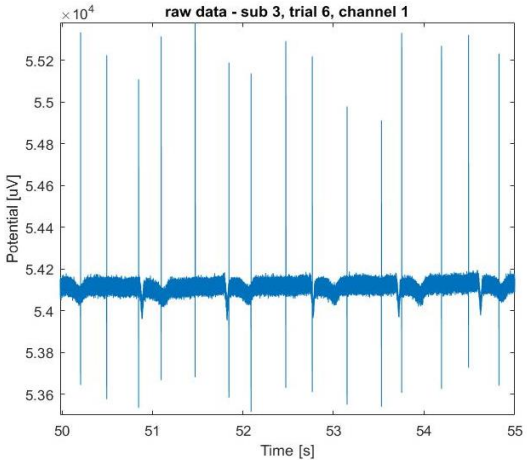

Figure 6: Close-up of the data in figure 5. An interval of 5 seconds reveals the presence of an ECG artefact (the small, regular negative peaks) and the presence of surface potentials elicited by the stimulations (the large, rapid, irregular peaks).

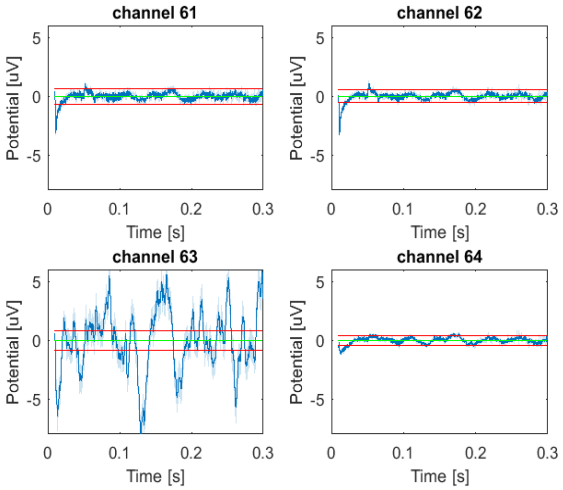

Figure 7: Averaged response to 1000 stimulations, as recorded by four adjacent electrodes concurrently. Electrical potentials regularly oscillated within the bounds of one baseline SD from the mean (channels 61,62,64). Sporadically, a channel presented with no such pattern (channel 63).

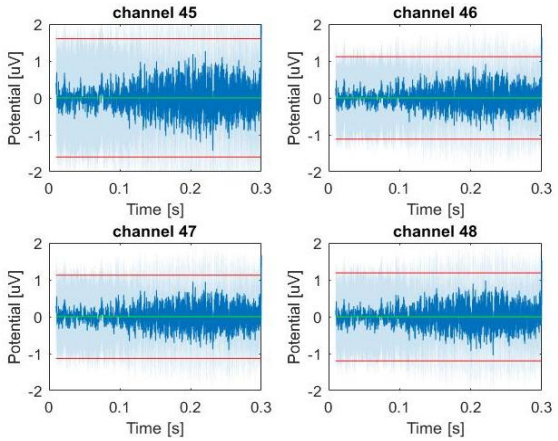

Figure 8: Typical averaged responses to the stimulations for recordings of subject 4. No SSEPs can be identified. High noise levels are evidently present, as demonstrated by the high baseline SD that occasionally exceeds 1.5 µV.

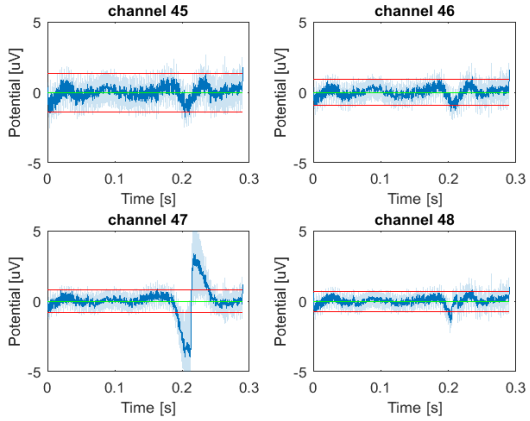

Figure 9: Averaged response to 1000 stimulations, as recorded by four adjacent electrodes concurrently. The centre green line represents the mean baseline as recorded by that channel. The red lines correspond to one SD (of the baseline) apart from said mean baseline. The dark blue and light blue data are the (filtered) stimulus-related average and its (original) SEM, respectively.

baseline served as a measure for the noise that is present in the response – see Figure 8.

Most of the channels presented little to no sign of a potential that originated at the stimulation site. However, this was not the case for every channel. The response to the stimulations, SSEP, was evidently present in multiple channels throughout the different subjects (excluding subject 4) and configurations.

In nine recordings, distributed over four subjects, SSEPs were present. In each of the three configurations there was at least one recording that presented SSEPs.

Configuration 1: Lateral of L4

The HD-sEMG grid that was located lateral (toward the side of the stimulated limb) of the L4 vertebra, was used to obtain ten recordings of evoked potentials. 154 Channels were considered erroneous and were discarded – see Table 1.

From the 486 remaining channels, 18 channels registered a clear SSEP – see Table 2. These SSEPs were registered during four different recordings. The time interval in which these peaks occurred was 0.131 to 0.245 s after the stimulation. The peak amplitude of the SSEPs ranged from 0.72 to 3.94 µV (1.88 ±0.88 µV). The average of the baseline and the average of the SDs of the baseline were, respectively, 0.00 and 0.67 µV, as measured by the same channels. Figure 10 presents a visualisation of an average SSEP in this configuration. The recorded SSEP and baseline values result in an average SNR of 5.23 (±3.56) dB. The highest SNR that was achieved, however, was larger than 12 dB.

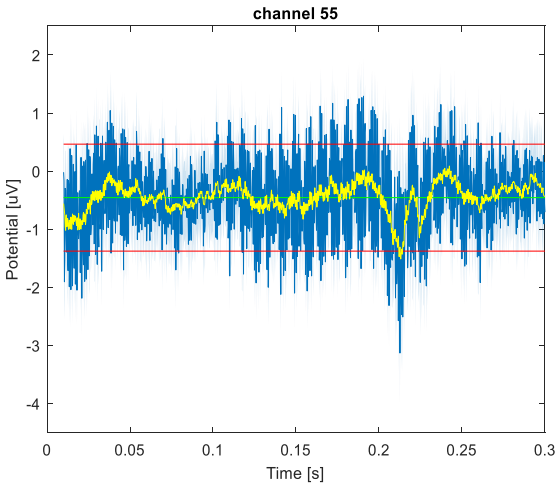

Figure 10: Stimulus-related averaged response of 1000 stimulations of the n. tibialis posterior, measured lateral of vertebra L4 using HD-sEMG electrodes. The green line represents the mean of the baseline. The red lines represent one SD of the baseline apart from its mean. The blue data is the SSEP, as registered by one channel. For visualization purposes, this data is Savitzky-Golay filtered and represented as the yellow line.

Table 1: Assessment of the averaged response to tibial nerve stimulation, as registered by each individual channel, resulted in the identification of erroneous recordings. Channels resulting in erroneous averaged responses were discarded.

Config. 1		Discarded channels	Config. 2		Discarded channels	Config. 3		Discarded channels
Sub-ject	Trial		Sub-ject	Trial		Sub-ject	Trial	
1	1	13,21,29,32,37,45,57	1	1	32	1	1	32
	2	29,32,37,45,57		2	32		2	32
2	1	32	2	1	1,5,32	2	1	32
	2	13,32		2	1,5,32		2	32
3	1	32	3	1	32	3	1	32,63
	2	32		2	5,9,17,18,21,29,32,37,45,53,61		2	32
4	1	1:64	4	1	1:64	4	1	1:64
	2	1:64		2	1:64		2	1:64
5	1	13,29,32,45,57	5	1	32	5	1	1,2,3,4,9,10,11,12,13,17,18,19,20,21,25,26,32,37,45,53
	2	29,32,37,57		2	32		2	32

To quantify the noise level that was present throughout the recording, the mean SD from the baseline is determined over all the channels that have not been discarded. The average SD from the baseline for this configuration was 0.68 µV.

The channels that registered the SSEPs, albeit in different trials, were channels: 3, 4, 10, 16, 24, 31, 40, 47, 48, and 55. Figure 4 shows the location of these channels in the electrode grid. Registration of SSEPs was more prevalent on the more medial side of the grid. This became also evident when examining the propagation of the potential over the grid – see Figure 11.

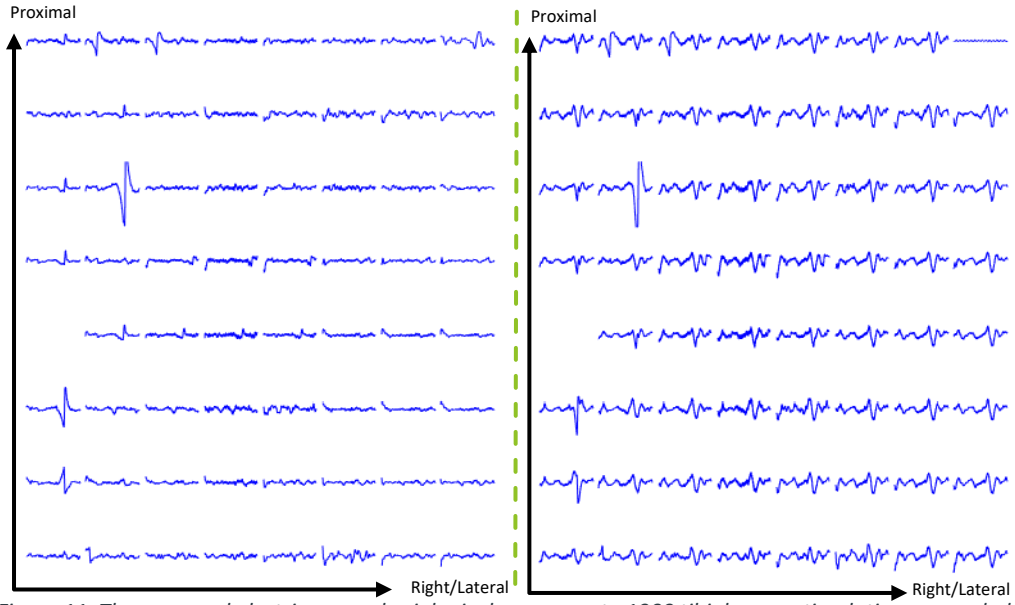

Figure 11: The averaged electric neurophysiological responses to 1000 tibial nerve stimulations, recorded on the skin surface lateral of vertebra L4, plotted for every channel for the time interval of 0.01 to 0.3 s following each stimulation. The left plot shows the 64 responses measured by the electrodes during trial 1 of subject 1. The right plot shows the 64 responses to the same stimulation protocol during trial 1 of subject 5.

Configuration 2: Central, L4

Also the second configuration was used to obtain ten recordings of evoked potentials. The recording site was central on the lower back at the level of the L4 vertebra. Out of the ten recordings, 150 channels were deemed erroneous – see Table 1.

Eight channels, out of the remaining 490, have registered a SSEP in this configuration (Table 2). All these SSEPs, however, were recorded in one subject during the same trail. The peaks of the evoked potentials presented themselves in the time interval from 141 to 210 ms after the stimulation. The average peak amplitude of the evoked potentials was 1.97 (±0.36) µV and ranged from 1.33 to 2.47 µV. The noise levels that were present in the recordings the SSEPs originated from were expressed in the average baseline mean, and the average baseline SD. These values were 0 and 0.77 µV respectively. The resulting average SNR for evoked potentials, measured centrally at L4 level, was 3.96 (±1.23) dB. The maximum value of the SNR that was obtained in this configuration was 6.56 dB.

The noise level in the recording of this configuration was greater than in the first configuration. The noise level in the second configuration were established to be 0.81 µV. Since the registrations of SSEPs was limited to eight channels in a single subject, no assumptions can be made with regard to the prevalence of stimulus-related potentials throughout the electrode grid.

Configuration 3: Central, Th12

Upon assessment of the averaged responses, it is evident that it is more complex in this configuration than in the other two configurations – Figures 11-13. It is also noted that SD of the response for this configuration is less than for the other two.

The number of recordings obtained in this configuration was equal to the previous two. After examination of each individual channel a total of 156 channels were removed. The signals from the remaining 484 channels were assessed for an evoked potential. This potential was identified in 16 cases. These SSEPs originated from three recordings distributed over two subjects – see Table 2.

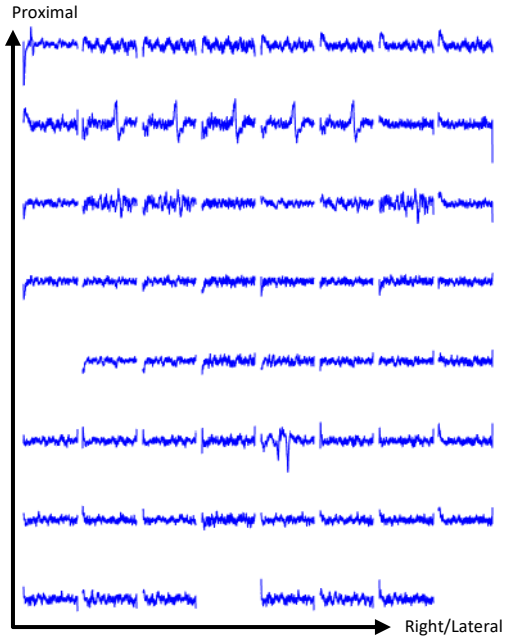

Figure 12: The averaged electric neurophysiological responses to 1000 tibial nerve stimulations, recorded on the skin surface centrally at the L4 vertebra, plotted for every channel of one trial for the time interval of 0.01 to 0.3 s following each stimulation. The SSEPs can only be identified in five channels.

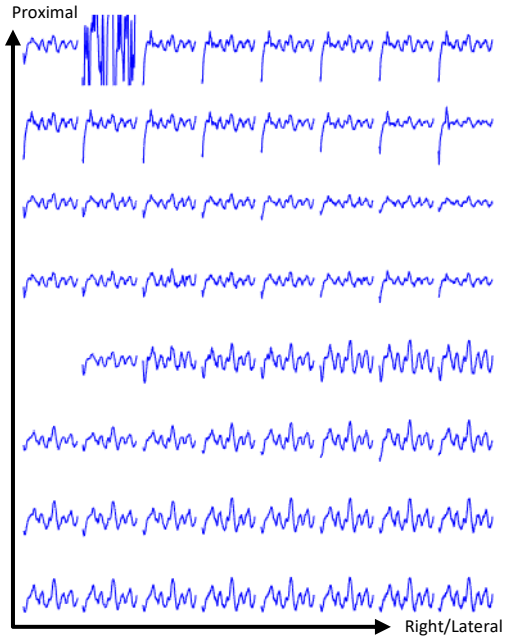

Figure 13: Alike figures 11 and 12, the averaged electric neurophysiological responses to 1000 stimulations, recorded on the skin surface. The plotted averaged responses were registered centrally at Th12 level during one trial. The averaged response presents itself in a complex pattern of multiple peaks.

The evoked potentials were registered in the time interval of 158 to 214 ms after stimulation. The peak amplitudes ranged from 0.39 to 0.77 µV and averaged 0.62 (±0.24) µV. The noise level in the channels that recorded the potentials were 0 and 0.33 µV, with the former being the baseline mean and the latter being the average SD of the baseline. This did not coincide with the overall noise level in the recordings of this configuration. The overall average noise level was determined to be 0.67 µV. Despite being in defiance with the noise level that was present in the channels that presented a SSEP, this was proportionate to the value of 0.68 µV, which was established to be the overall noise level of the first configuration.

The SNR for the 16 identified potentials averaged at 3.96 (±2.03) dB. The highest SNR that was acquired in this configuration was a SNR of 8.49 dB.

Contrary to configuration 1, the identified SSEPs provided insufficient insight to make assumptions regarding the prevalence throughout the grid.

Table 2: Signal-to-noise ratios, peak amplitudes, and time intervals of somatosensory evoked potentials that were identified in healthy, young adults, after 1000 tibial nerve stimulations.

Recording			Channel	SNR [dB]	PeakAmp	T_{int} [s]
Subject	Config.	Trial			[µV]	$t_{stimulation}$ = 0.00
1	1	1	3,4,16,24,31,40,47,48,55	4.04 ±3.75	1.81 ±0.93	0.131-0.238
		2	-	-	-	-
	2	1	-	-	-	-
		2	-	-	-	-
	3	1	42	4.96	1.46	0.158-0.161
		2	-	-	-	-
2	1	1	-	-	-	-
		2	28	6.56	2.47	0.167-0.171
	2	1	20,42,47,51,52,53,54, 55	3.96 ±1.23	1.97 ±0.36	0.141-0.210
		2	-	-	-	-
	3	1	-	-	-	-
		2	-	-	-	-
3	1	1	10	12.16	2.03	0.190-0.204
		2	-	-	-	-
	2	1	-	-	-	-
		2	-	-	-	-
	3	1	12,14,15,16,34,35,39, 40,42,47,49,50	2.93 ±0.65	0.54 ±0.09	0.162-0.176
		2	14,15,49	7.73 ±0.66	0.68 ±0.08	0.195-0.214
5	1	1	16,24,40,47,48,55	6.00 ±1.93	2.22 ±0.90	0.189-0.238
		2	3	8.09	1.34	0.243-0.245
	2	1	-	-	-	-
		2	-	-	-	-
	3	1	-	-	-	-
		2	-	-	-	-

Discussion

Examination of the neurophysiological activity of the spinal cord is currently realized by using needle EMG. This is an invasive test that can be particularly painful and is not free of risk. The aim of this study was to determine whether HD-sEMG is adequately equipped for registering neurophysiological activity in the spinal cord in a non-invasive manner. If it is indeed the case that HD-sEMG is a viable technique for the assessment of neurophysiological activity, it has the potential to be more reliable and aid in the earlier diagnosis of, among other things, neuromuscular disorders.

Somatosensory evoked potentials were assessed for their signal-to-noise ratio to determine whether a response could be objectively identified. In all the tested configurations peak-to-peak amplitude of the stimulus response exceeded the significance interval defined via a baseline recording (resting state). This led to the conclusion that HD-sEMG is indeed a valid tool for assessing nervous activity in the spinal cord.

Reliable activity patterns and evoked potentials could be identified (Figure 8). For configuration 1, the findings were conform with the pilot conducted by Luger & Daffertshofer (2016). Additionally, in this study the possibility to reliably identify evoked potentials is demonstrated for all three configurations. However, not all three configurations reported the same degree of reliability. Configuration 1 provided the highest SNR. Its average SNR was found to be 5.23 (±3.56) dB. Configuration 2 and 3 both produced a SNR of 3.96 dB, the SD of configuration 2 and 3 were ±1.23 and ±2.03 dB, respectively. This was found to be sufficiently high to suggest HD-sEMG as a potential, non-invasive, alternative technique for assessment of the neurological activity in the spinal cord. Only configuration 1 presented with enough information to suspect evoked potentials to be more prevalent throughout the medial side of the grid.

Limitations of current study

Although the SNRs reported in this study indicate the ratio between the desired signal and the noise correctly, they are of limited usefulness. The reason for this is their lack of statistical meaning. It would be fallacious to derive a confidence interval from the SNRs. The calculation of the SNR as done in this study resembles that of the t-statistic, which gives an indication of the confidence interval. However, they are not equal. Recall equation (1) served for determining the SNR where P_{signal} is the average power of the signal, and P_{noise} is the average power of the noise. P_{noise} is derived from the A_{noise}, which is the SEM of the baseline. This causes P_{noise} to be the SEM of the baseline expressed as power. Since P_{noise} is the SEM of the baseline and P_{signal} is the average of the desired signal (both expressed as power), equation (1) can be reformulated

$$SNR = \frac{\bar{X}}{SEM_{\mu}} \qquad (6)$$

where $\bar{X}$ is the average of the desired signal and SEM_{μ} is the SEM of the baseline. The desired signal is actually the difference between the measured signal and the mean of the baseline, although the latter is assumed to be 0. When this is added to equation (6), the following equation arises

$$SNR = \frac{\bar{X}-\mu_0}{SEM_{\mu}} \qquad (7)$$

where μ_0 is the mean of the baseline. When compared to the equation for the t-statistic, the resemblance is evident.

$$t = \frac{\bar{X}-\mu_0}{SEM_X} \qquad (8)$$

where t is the t-statistic, $\bar{X}$ is the sample mean, μ_0 is the population mean the sample is tested for, and SEM_X is the SEM of the sample. Since the decision was made to use the baseline SEM instead of the signal SEM to better evaluate the noise levels, the SNR provides no statistical evidence. Hence, the reported SNRs strongly suggest that HD-sEMG can be a viable, non-invasive alternative for needle EMG, but provides no statistical evidence to support it. Despite the absence of statistical evidence, the SNRs that were found are promising. Further research is recommended.

There are multiple aspects of this study where future research could improve on. First and foremost, to give credibility to the potential of HD-sEMG as non-invasive assessment spinal functioning, statistical evidence is required. This study presents evidence that it is possible to achieve high SNRs, suggesting that the

magnitude of the evoked potential is also substantial. It is therefore expected that the required sample size to achieve an objective statistical power is fairly limited.

Another limitation to the reliability of this study is caused by the baseline. To compare the aligned and averaged epochs of the response, 1,000 epochs are randomly created in the baseline signal. However, this signal was not long enough to create these time intervals without overlap. As a result, the 1,000 intervals used for the baseline were not fully independent, which could lead to a biased result. The impact this had on the outcome parameters is arguably small.

The final limitation of this study is the subjective examination for erroneous channels. Strict criteria for exclusion were absent, calling for a subjective judgement on the quality of each channel. Impedance measurements during the recording could offer more objective criteria for exclusion.

Recommendations for further research

There are also multiple directions further research could go to form a more comprehensive view of the possibilities HD-sEMG offers for the assessment of spinal functioning. The current study focused on three different locations on the lower back. There was, however, no evidence to suggest the evoked potentials were less likely to be identified at Th12 than at L4 level. This raises the question; how proximal might the SSEPs be detectable? If multiple grids are used simultaneously, for instance as a strip along the spine, one must realize the amount of data that is generated. Nonetheless, such an experimental set up might prove the potential that HD-sEMG has to measure SSEPS. In addition, such an experimental set up could also establish its effectiveness for measuring neural propagation.

Another direction further research could take is the in vivo mapping of the individual neural pathways. Applying geometry to the differences in signal intensity and phase of the signal could potentially allow for mapping of individual neural pathways. Configuration 1 already presented information to suspect that evoked potentials are inclined to be present at fixed locations, supporting the hypothesis that the spatial origin can be identified via surface recording.

A final recommendation for further research is to increase the number of stimulations in the stimulation protocol. Despite numerous clearly identified SSEPs, the consistency of the standing wave across the electrode grid was seldom evident. Moreover, the SSEPs that were apparent in a certain channel during a trial were wanting the subsequent trial. A higher number of stimulations might resolve this, since it would decrease the level of noise disproportionately to the signal.

Conclusion

The aim of this study was to determine the viability of HD-sEMG as a technique for registering neurophysiological activity in the spinal cord in a non-invasive manner. Recordings on three different locations on the lower back were obtained during electro stimulation of a peripheral nerve. These recordings resulted in more or less pronounced stimulus-related potentials. Conform with the findings of Luger & Daffertshofer (2016), a reliable activity pattern was detected just lateral of the L4 vertebra. However, also on top of the L4 and Th12 vertebra were somatosensory evoked potentials clearly present. The average signal-to-noise ratios of the identified evoked potentials were respectively: 5.23 (±3.56), 3.96 (±1.23), and 3.96 (±2.03) dB. Although statistical evidence for these findings is absent, it is a firm suggestion that HD-sEMG is indeed viable for the monitoring of spinal function.

For future research, it is recommended to supply statistical evidence to prove the viability of HD-sEMG to monitor spinal functioning. Furthermore, a more extended stimulation protocol is required to improve the consistency throughout the recordings. Moreover, the results suggest no indication of a decreased capability of identifying evoked potentials. It is, therefore, recommended to study the range of the spine where HD-sEMG can be used. Additionally, early evidence seems to suggest that the spatial location of the potential can be identified using HD-sEMG. This raises the question whether HD-sEMG recordings can be combined with geometric models to map out the neurological pathways in the spinal cord.

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Appendix

Appendix I: Projectplan

Introduction

Diagnostics of neuropathies of the spinal cord

The current procedures for assessing neural activity and SSEPs in the spine all have certain shortcomings. The most common assessment of the spinal cord is done through physical examination. Doctors can recognize spinal cord disorders based on certain symptoms, such as: localized numbness, (partial) paralysis, and pain. However, since many of these disorders have symptoms in common, it is often necessary to explore the origin of the symptoms further using more advanced techniques.

MRI is the most common imaging test for spinal cord disorders. It shows abnormalities in the soft tissues around the cord, such as: abscesses, hematomas, tumors, and ruptured disks. MRI can also detect anomalies in the bone surrounding the spinal cord, revealing: tumors, fractures, and cervical spondylosis. MRI has its limitations for assessing the spinal cord. Registering spinal activity itself is out of the scope of a MRI. Although brain activity can be recorded using fMRI, the absence of blood flow in the spinal fluid prevents this for the spinal cord. Furthermore, a MRI is expensive and metal or implanted devices (pacemakers) prohibit the use of the MRI. The alternative is myeolography; CT with a contrast agent. It is less costly than MRI, but also less detailed and unable to detect spinal activity. For assessment of the spinal cord, it is required to inject the contrast agent in the subarachnoid space.

To assess the function of the spinal cord, the neural activity needs to be registered. The technique that is most commonly used for this purpose, is needle EMG. It is an invasive procedure where needles are stung in the subarachnoid space to detect the electric potentials of the spinal cord (Desmedt & Cheron, 1980). This provides a highly detailed measurement of the spinal activity.

Somatosensory Evoked Potentials (SSEPs)

Since a large variation of potentials can occur in the spinal cord, the sole registration of these potentials yields insufficient information to confirm a diagnosis. A standardized method is used to overcome this. A peripheral nerve is stimulated via an electro-stimulation protocol. The neural response to each stimulation, a standing wave, travels through the nerve and via the spinal cord to the brain. These Somatosensory Evoked Potentials (SSEPs) can be registered in the spinal cord (Nuwer, 1998). The measured SSEPs and their moments of origin are combined to form the complete picture of the functioning of the spinal cord. The SSEPs are very sensitive to impairment. As a result, this method can meet a variety of specific clinical objectives (Chawla, Burneo, & Barkley, 2016):

7. To establish objective evidence of abnormality.
8. To look for clinically silent lesions.
9. To define an anatomical level of impairment along a pathway.
10. To provide evidence about the general category of the pathology.
11. To objectively monitor changes in the patients status over time.

Intraoperative monitoring of the SSEPs is nowadays most commonly used to safeguard the central nervous system during high risk surgeries. Continuous monitoring can warn a surgeon and prompt intervention before impairment becomes permanent.

SSEPs tests

As mentioned before, needle EMG is the conventional procedure for measuring the SSEPs. The main shortcoming with this test is its invasiveness. A needle to the subarachnoid space of the spinal cord can be painful and it is not risk free. The spinal cord is highly sensitive and damage to it can have far stretching consequences. An alternative technique to register the SSEPs in the spinal cord, that is non-invasive and risk free, is desired.

HD-sEMG as alternative procedure

The alternative for needle EMG to measure muscle activity is surface EMG (sEMG). The common bipolar sEMG proved to be insufficiently equipped to provide accurate information on muscle and neural activity for diagnostic purposes (Linsen, et al., 1991). However, High-Density sEMG (HD-sEMG) is believed to be adequately equipped to register the spinal activity. At the VU department of Human Movement Sciences, an initial pilot study has been conducted to detect neural activity of the spinal cord after peripheral electro-stimulation of the n. tibialis posterior (Luger & Daffertshofer, 2016). Using HD-sEMG grids above lumber vertebrae L4 and L5 reliable activity patterns have been found with a high signal-to-noise ratio (S/N-ratio) after 1,000 stimuli. However, the spatial coverage during this pilot study was about 8 cm and the ADC involved was limited to a sampling rate of 2048 HZ. It remains to be objectively confirmed that the signal recorded during this pilot was indeed neural activity in the spine, not neural activity from the plexus lumbalis or dorsal root of the sciatic nerve. If this is the case, it would hence confirm HD-sEMG as a reliable and non-invasive test for SSEPs. The aim of this study is to determine whether HD-sEMG is indeed adequately equipped to register SSEPs in the spinal cord. The neural activity is examined on three locations on the lower back and state-of-the-art HD-sEMG equipment is used with a sampling rate of 16.384 Hz and a stimulation protocol of 1.000 stimuli to the tibial nerve.

Method

Subjects

As in the aforementioned pilot recordings by Luger & Daffertshofer (2016), (up to) ten young (18-30) and healthy adult subjects will participate in the current study

Protocol

Before the experiment is started, the subject will be informed of, and prepared for, the experiment. The subjects will lie in a prone position during the course of the experiment. The Posterior tibial nerve[iii] (N. Tibialis Posterior) will be stimulated. The resulting neural activity will be recorded with HD-sEMG grids at 3 different sites on the lower back.

Preparation

Prior to the application of the electrodes, the skin of the subject will be shaven and cleansed with alcohol. This will minimalize impedance between the skin and the electrode, which will decrease the noise in the measured signal.[iii] After the cleansing of the skin, the electrodes for the electro-stimulation system (Dura Stick pads) and the HD-sEMG system will be placed. The cathode for the stimulation is placed between the medial border of the Achilles tendon and the posterior border of the medial malleolus. The anode is located 3 cm in distal direction.[iv] A ground electrode is placed on the caput fibula of the same limb to reduce stimulus artefact. There are 3 configurations for the placement of the 64-channel HD-sEMG grid (8x8 electrodes, 4mm inter-electrode distance):

4. Lateral of the processus spinosis of the L4 vertebra to the stimulated side. The median edge of the electrode grid touching the midline of the back.[v]
5. Over the L4 processus spinosis.[vi]
6. Over the Th12 processus spinosis.[vii]

Each of these 3 configurations is accompanied by a reference electrode located 2 cm lateral of the electrode grid in the direction of the non-stimulation side.

Stimulation

The peripheral n. tibialis posterior is transcutaneously stimulated. Monophasic rectangular pulses with a 0.2 μs duration are delivered at a rate of ±3 Hz.[viii] The stimulation protocol consists of 1000 stimulations. It is pseudo-randomised to prevent habituation. The protocol will be applied twice for each HD-sEMG grid configuration. The stimulus intensity needs to be adequate to produce a consistent, but tolerable, muscle twitch in the subject's foot or toes. This is likely to be around 30-45 mA, since a constant voltage stimulator is used.

Recording
Contact impedance of the surface electrodes should be kept at less than 5 KΩ. The HD-sEMG amplifier records the 64 channels of the HD-sEMG grid, the reference and ground electrode, and the trigger from the stimulator. Its sampling rate is set at 16.384 Hz (2^{14} Hz). Each configuration of the HD-sEMG electrodes is measured separately. This results in 6 measurements of approximately 5 minutes and 30 seconds per subject.

Data processing
The recorded signal will be offline high-pass filtered with a cut-off frequency of 10 Hz (2^{nd} order Butterworth). ECG artifacts will be removed using principal component analysis. The trigger from the stimulator is used to determine epochs in the recording. The epochs are aligned and averaged to obtain the SSEPs for each of the 64 recorded sites. The resulting stimulus-related averages will be assessed for the S/N-ratio (Signal-to-Noise Ratio).

[ii]

[ii] Responses to posterior tibial nerve stimulation are subject to less intersubject variability than those to common peroneal nerve stimulation (Pelosi, Cracco, Cracco, & Hassan, 1998).
[iii] Lowering the impedance decreases the noise in the signal. Subsequently, the S/N-ratio increases. The number of trial is unchanged. Ergo, the statistical power increases (Kappenman & Luck, 2010).
[iv] In accordance with the AEEGS guidelines (American EEG Society, 1994).
[v] This configuration records the activity of the sensory (dorsal) root.
[vi] This configuration records the neural activity of the cauda equina, inside the spine.
[vii] The conus medullaris, the most distal part of the spinal cord, is located at the level of L1, L2, or lower. Hence, this configuration records the neural activity of the actual spinal cord.
[viii] A rate of 3-6 Hz is recommended. However, exact subharmonics the line frequency (50 Hz) should be avoided. These would lead to contamination with large artifacts of the 50 Hz frequency in the averaged SSEPs (Legatt, 2014).

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