The use of high-density EEG to map out cortical motor activity and reorganization following lower-limb amputation

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Abstract

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Introduction

Studies have shown that after amputation, changes occur in the sensory and motor cortex. These changes are called cortical reorganization, where adjacent cortical areas occupy the cortical area of the amputated limb. High-density electroencephalography (EEG) has been used to observe cortical reorganization in the motor cortex following upper limb amputation. The aim of this study was to use high-density EEG to map out motor cortical activity and cortical reorganization following lower limb amputation.

Materials and methods

One healthy and one left transfemoral amputee participated in the study. Using a 256-electrode, high-density EEG system, EEG signals were acquired while participants performed sets of motor tasks. The amputated participant was asked to try to voluntarily execute each movement for the missing limb, not simply imagine the movement. EMG data was acquired simultaneously. Participants walked on a treadmill for four minutes while EEG and EMG data was recorded. The EEG data was mapped onto structural MRI brain images and motor activity generated by each set of movement was localized in the motor cortex. Additionally, diffusion tensor imaging analysis of neural tracts was performed.

Results

By mapping the cortical surface potential over time, cortical activity was observed in different parts of the brain, e.g. the visual cortex, prefrontal cortex and the motor cortex. For the amputated participant, motor representations for movement tasks were mapped out and showed additional cortical activity located more laterally than expected from somatotopic maps of the motor cortical homunculus. This additional cortical activity was not observed for the healthy participant.

Discussion

The results indicate that motor cortical activity and cortical reorganization following lower limb amputation can be observed using high-density EEG. More measurements and data analysis is required, e.g. with the use of other processing software. The diffusion tensor images show neural tracts but further work is needed to evaluate whether white matter changes associated with the amputation can be observed. The method could be used to evaluate the effects of high-tech myoelectric prostheses with sensory feedback on cortical reorganization. It would also be interesting to further investigate the connection between cortical reorganization and phantom limb pain.

Ágrip

Notkun há-þéttleika heilarafrits (EEG) til að kortleggja virkni hreyfibarkar og endurröðun hans í kjölfar aflimunar neðri útlims

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Inngangur

Rannsóknir sýna að eftir aflimun verða breytingar á svæðum í heilaberki. Þessar breytingar kallast endurskipulagning (e. cortical reorganization) og gerast bæði í skynberki og hreyfiberki, þar sem heilasvæði aðlægra líkamshluta þenjast inn í svæði aflimaða líkamshlutans. Há-þéttleika heilarafrit (e. EEG) hefur verið notað til þess að meta endurskipulagninu hreyfibarkar eftir aflimun á efri útlim. Markmið rannsóknarinnar var að kanna hvort hægt væri að nota há-þéttleika heilarafrit til þess að kortleggja heilavirkni í hreyfiberki og meta endurskipulagningu í kjölfar aflimunar á neðri útlim.

Efniviður og aðferðir

Þátttakendur voru tveir, einn með aflimun við lærlegg og einn án aflimunar. Há-þéttleika heilarafrit með 256 rafskautum var notað til að mæla heilavirkni á meðan þátttakendur framkvæmdu mismunandi endurteknar hreyfingar á neðri útlimum. Þátttakandinn með aflimun var beðinn um að reyna meðvitað að framkvæma hreyfingarnar þeim megin sem aflimunin er en ekki bara ímynda sér hreyfingarnar. Vöðvarafrit (e. EMG) mældi virkni vöðva samtímis. Þátttakendur gengu á hlaupabretti í fjórar mínútur á meðan heilarafrit og vöðvarafrit voru mæld. Segulómmynd (e. MRI) af heila var tekin af þátttakendum. Staðsetning heilavirkni fyrir hverja hreyfingu var reiknuð út frá heilarafritsmerkjunum og segulómsmyndinni. Að auki var diffusion tensor myndtækni (e. Diffusion tensor imaging) beitt til þess að kortleggja taugabrautir í heila þátttakenda.

Niðurstöður

Kortlagning yfirborðsspennu yfir tíma sýndi virkni í heilaberki frá sjónberki að hreyfiberki. Hjá þátttakandanum með aflimun sást auka virkni í hreyfiberki hliðlægt við fótleggjasvæði í hreyfiberki sem ekki sást í þátttakandanum án aflimunar.

Ályktanir

Niðurstöðurnar benda til þess að hægt sé að rannsaka endurskipulagninu í hreyfiberki eftir aflimun á neðri útlim með há-þéttleika EEG. Þörf er á frekari mælingum og úrvinnslu, t.d. með nýjum úrvinnsluforritum. DTI aðferðin sýnir corticospinal taugabrautirnar en meiri vinnu þarf til að sjá hvort um rýrnun sé að ræða þeim megin sem tilheyrir aflimuninni. Aðferðin gæti verið notuð til að meta áhrif hátækni gerviútlima sem er stjórnað af vöðvavirkni og veita notendum upplýsingar um afstöðu útlimsins (e. sensory feedback). Einnig væri áhugavert að skoða tengsl draugaverkja og endurskipulagningar en magn endurskipulagningar sem á sér stað hefur verið tengt við styrk draugaverkja.

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Abbreviations

BOLD	Blood-oxygen-level dependent
DTI	Diffusion tensor imaging
EEG	Electroencephalography
EMG	Electromyography
FA	Flip angle
fMRI	Functional magnetic resonance imaging
FOV	Field of view
GLEAS	The Global Lower Extremity Amputation Study
IMES	Implanted myoelectric sensors
LTD	Long term depression
LTP	Long term potentiation
PET	Positron emission tomography
RANNÍS	Rannsóknarmiðstöð Íslands
SE-EPI	Spin echo – echo planar imaging
TE	Time to echo
TI	Inversion time
TR	Time repetition

1 Introduction

Previous studies have demonstrated reorganization in the sensory and motor cortex after limb amputation (1-4). Cortical neuroplasticity after amputation of a limb has been evidenced by a number of methods, e.g. positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (5, 6). A recent study showed cortical reorganization in upper limb amputees using high-density electroencephalography (EEG) (1).

1.1 Amputation

The loss of limb by amputation is a traumatizing experience and has a deep impact on both physical and mental health. Whether caused by accident, to treat peripheral vascular disease, neurological injury or any other reason, amputation has been linked with decreased quality of life (7). Amputations can be classified into upper and lower amputations; moreover they can be classified into major and minor amputations. Generally, major amputations impact the quality of life more than minor amputations. One study found that of all individuals with a loss of limb in 2005 in the USA, a total of 65 % had undergone lower limb amputation (8).

1.1.1 Lower limb amputation

The most common cause of lower limb amputation are non-healing foot ulcers, commonly caused by diabetes, infection or peripheral vascular disease (9). Minor lower limb amputations can be defined as amputation distal to the ankle joint, whereas major amputations are through or proximal to the ankle joint (10). The most common major amputations performed are below-knee (transtibial) and above-knee (transfemoral) amputations (11).

1.1.2 Epidemiology of lower limb amputation

Incidence of lower limb amputation varies around the world. The Global Lower Extremity Amputation Study (GLEAS), published in 2000, compared incidence rates of lower extremity amputation around the world. Data was collected from ten medical centers in six countries, England, United States, Italy, Spain, Taiwan and Japan, all with populations greater than 200.000. The study found differences in overall incidence of amputation and there was a tenfold difference between the highest area and the lowest area. The incidence rates per year for first major amputation in men ranged from 2.8 per 100.000 population in Madrid, Spain to 43.9 per 100.000 population in the Navajo population, United States (12). Another study compared the non-U.S. data from the GLEAS study to data from 12 U.S. counties. The age-adjusted incidence of major amputations in the U.S. medical centers was 25.6 per 100.000 per year while the non-U.S. centers reported an average of 14.2 per 100.000 population for men. The major amputation rate for women was also higher in the U.S (13).

Most lower limb amputations are major. A study on limb amputation prevalence in the USA in 2005 found that out of all lower limb amputations, over one half were major. Overall, the prevalence of minor lower limb amputations was 404.000 but the prevalence of major lower limb amputations was 623.000.

Conversely, most upper limb amputations are minor which are defined as loss of fingers or hands. In the study, the prevalence of minor upper limb amputations was 500.000 and the prevalence of major upper limb amputations was 41.000 (8). From 2000-2013, a total of 214 patients underwent major lower limb amputation in Iceland (14).

1.2 Brain reorganization after peripheral deafferentation

1.2.1 Brain plasticity

The brains ability to change and develop through a lifetime is called brain plasticity. For a long period of time many researchers believed that the brain only developed functionally and anatomically during childhood and the adult brain was unable to change in that way. Recent progress in the field of neuroscience has established that the adult brain is plastic; plasticity is a continuous state, the brain is constantly being modified by changes in setting and situations (15). It has been demonstrated that structural changes occur in the brain as a response to change in behavior (16-18). Studies have shown training-dependent changes in brain matter, e.g. increased gray matter volume in professional musicians compared to non-musicians (19) and increased brain volume in aging humans after only six months of regular aerobic training (20). There is also evidence for plasticity at the subcortical, level including the spinal cord, brainstem and thalamus (2). At the subcortical level, correlation has been found between volume in the hippocampus and navigational experience in taxi drivers. The study found that taxi drivers had a larger posterior hippocampus than the controls (21).

1.2.2 Mechanisms of brain plasticity

Several mechanisms are believed to be responsible for brain plasticity. In 1949, Donald Hebb introduced the Hebbian theory which states that repeated neuronal firing may encourage growth or metabolic change that strengthens a neuronal synapse (22). To date, this synaptic strengthening is believed to play a fundamental role in brain plasticity. In 1973, Bliss and Lømo demonstrated long term potentiation (LTP) by repetitive stimulation of the perforant path of the hippocampus in the rabbit brain (23). LTP is the action-dependent strengthening of synapses and is an essential mechanism in memory and learning. In contrast to LTP, long term depression (LTD) is the weakening of efficacy of neuronal synapses after repetitive stimulus (24). Another process, the growth of new axon terminals and formation of new synapses (synaptogenesis) after LTP contributes as another mechanism of the plastic brain (25). Along with the potential formation of new synapses, morphological changes at previously existing synapses occur (26). Neurogenesis, one more process believed to contribute to brain plasticity, is the process in which neurons are generated from progenitor cells. In the mammalian brain, adult neurogenesis is believed to exist in a few regions of the brain, mostly in the dentate gyrus of the hippocampus and the olfactory bulb (27).

These processes seem to be engaging at different times (28) and are non-autonomous to each other. LTP and LTD are intertwined where LTP at one synapse might promote LTD at a neighboring synapse. Subsequent structural changes appear where LTP can induce synaptic growth while LTD can cause deprivation of synapses. Furthermore, newborn neurons seem to be hypersensitive to synaptic plasticity and better suited to undergo LTP than adult neurons (29).

1.2.3 Cortical homunculus

A physical representation of the human body can be found in both the somatosensory cortex and the motor cortex of brain and is called the cortical homunculus. For example, sensory information from the hand projects contralaterally to one site in the somatosensory cortex, whilst the foot projects to another. Penfield and Boldrey first described this in a paper in 1937 (30). This was the first visual way to depict the cortical map (31). The representations of the foot, leg and hip are located at the top of the cerebral hemisphere, where the trunk and upper limb are represented more laterally while representations of the face, mouth, lips and tongue lie closest to the lateral sulcus (31). Since they were first described by Penfield and Boldrey, multiple studies using modern neuroimaging methods have reproduced these results (32). Although a basic topographical layout exists, the organization in the cortex is not clearly differentiated and considerable overlapping occurs in cortical areas (33).

1.2.4 Cortical reorganization following peripheral injury

The cortical representation of various body parts constantly changes based on the pattern of afferent nerve impulses (34) and if a cortical area is deprived of its input, e.g. after an amputation, adjacent cortical areas of the sensory cortex will occupy the silent areas (35). When specific movements of the phantom limb are attempted, the cortical area of the phantom limb may move out of the original area into bordering cortical areas for other body parts (36). This is called cortical reorganization and has been evidenced by multiple studies for the sensory and motor cortex (1-4). The effect of deafferentation following peripheral injury is apparent at the molecular and systematic level (37). Cortical reorganization occurs following any means of deafferentation, e.g. amputation and even by local cutaneous anaesthesia for the somatosensory cortex (38). In a study of 10 healthy volunteers, MRI data showed that rapid cortical reorganization occurs in the somatosensory cortex after local cutaneous anaesthesia. After applying EMLA cream to the right forearm, cortical reorganization was observed in the contralateral somatosensory cortex as the right hand area expanded over the anaesthetised right forearm area. Using a Semmes-Weinstein monofilament, thresholds for touch/pressure at digits II and V were assessed as well as tactile discrimination using static two-point discrimination. A significant improvement in tactile discrimination of the right hand was documented. The authors presumed that the sensitivity improvement was a result of the expansion of the hand area in the somatosensory cortex where more nerve cells were made accessible for the hand (38).

1.2.5 Methods for observing cortical reorganization

Cortical reorganization after limb amputation has been evidenced by a number of neuroimaging methods, e.g. positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (5, 6). High density EEG has also been used to show cortical reorganization following amputation in the upper limb (1, 39) and to the best of our knowledge, this has not been shown for lower limb amputation.

Functional magnetic resonance imaging (fMRI) is a haemodynamic method that detects changes in the blood flow in the brain. fMRI is a non-invasive imaging technique that doesn't involve radiation (40). The most commonly used method is based on spotting the blood-oxygen-level dependent (BOLD) contrast in the brain so it can be used to map out neural activity in relation to energy used by brain cells (41). The concept, BOLD contrast, was introduced in 1990 by Ogawa and colleagues (42) where they demonstrated a method that could track blood oxygenation changes in the brain. Oxygenated and deoxygenated hemoglobin has different magnetic properties which can be used to track neural activity (43). fMRI can be used to localize brain activity with high accuracy (44) and fMRI has been used to observe cortical reorganization in multiple studies (45-48).

Positron emission tomography (PET) is another method used to study neural activity and has been used to study cortical reorganization and brain plasticity (49, 50). Three-dimensional images are constructed using radioactive tracers injected into the blood stream. The tracer is a biologically active molecule that is chosen according to what trait is to be studied, e.g. for cerebral blood flow, the tracer is $H_2{}^{15}O$ is used but for glucose metabolism, fludeoxyglucose (^{18}F) is used (51). Although PET is an important method in research and diagnostic medicine, using radioactive isotopes is a drawback because of health concerns (52).

Electroencephalography (EEG) is a noninvasive electrophysiological method for monitoring brain activity. Multiple electrodes are placed on the scalp for recording the electrical activity of the brain over a period of time (53). Pyramidal neurons of the cortex are the main source of the EEG signal and EEG is less sensitive to signals originating deep in the brain (54). EEG measures the electrical potential generated by thousands of millions of neurons that have similar spatial orientation. EEG cannot measure the activity of a single neuron. EEG data can be combined with anatomical images, such as MRI, and electrophysiological brain activity mapped onto anatomical landmarks (54).

As discussed above, both fMRI and PET have been extensively used to study functional neuroanatomy (55) but EEG has potential advantages over these methods. fMRI and PET have time resolutions of between seconds and minutes whilst EEG can have a time resolution in the sub millisecond-range. EEG can also be used to monitor moving patients in real time while this is much more difficult with the other methods requiring immobile equipment (53). EEG has its disadvantages, e.g. EEG has lower spatial resolution than fMRI (54).

In many conventional EEG measurements, 21 channel EEG recordings are used, but up to 256 electrodes can be used to increase spatial resolution and localization in a particular area, e.g. the somatosensory or the motor cortex of the brain (56-59). In Iceland, as part of a RANNÍS sponsored multi-institution project, a high-density system was recently acquired that was used in this study.

1.2.6 Neural tract diffusion tensor imaging

Diffusion tensor imaging (DTI) is a magnetic resonance imaging technique that uses the pattern of water molecule diffusion in tissue for the production of white matter images. DTI is used in neural tractography and fractional anisotrophy is a common property to describe during DTI analysis (60). DTI has been used to observe neuroplastic changes in neural tracts following limb amputation (60). A study on lower limb amputees presented decreased fractional anisotrophy, a measure of diffusion of water in a voxel, in the corona radiata along with microstructural changes in commissural fibers connecting the bilateral premotor cortices (61). In another study, a reduction in fractional anisotrophy has been observed bilaterally in the corpus callosum following lower limb amputation (62).

1.2.7 Surface electromyography

Surface electromyography (EMG) is a non-invasive technique used to study muscle function through electrical activity. By using electrodes, this method can detect electrical potential generated by skeletal muscle cells. EMG is used to test a variety of disorders of neuromuscular nature. The use of EMG is ideal to evaluate the characteristics of potentials in voluntary movement or potentials when the patient is at rest. It can be used to measure the on and off switching of muscles (63). Surface electromyography can be used simultaneously with high-density EEG, which allows the researcher to observe and connect muscle activity with cortical activation.

1.3 Targeted reinnervation

Targeted reinnervation is a surgical procedure where the inactivated, residual sensorimotor nerves, previously responsible for innervating the missing limb, are surgically re-routed to alternative denervated muscle groups and skin areas. Once reinnervated, the patient of the missing limb is able to contract the nearby-reinnervated muscle through the original motor efferents that had been cut by the amputation. In a similar manner, the reinnervated sensory afferents are able to provide sensory feedback for the missing limb via the skin (64).

The first performed surgery for the targeted muscle reinnervation for improved myoelectric control was performed in 2004, in a patient with bilateral amputations at shoulder disarticulation level. In the surgery, the nerves from the brachial plexus were transferred into the pectoralis muscles. The musculocutaneous nerve was transferred to the upper pectoralis major, the median nerve was transferred to the middle pectoralis major and the radial was transferred to the lower pectoralis major. The fourth nerve, ulnar nerve, was transferred to the pectoralis minor muscle (65). The pectoralis muscles had been denervated and the original nerves prevented from reinnervating the muscle. About three months later, the patient experienced the first voluntary twitch in his pectoralis major when imagining bending his elbow. In five months, he was able to contract three different locations on the pectoralis major muscle attempting different motions and three independent EMG signals were recorded over the musculocutaneous and the median nerve innervation locations. Sensory information had also been reinnervated so when the patient was touched on different parts of the chest, he felt sensations in the arm and hand (65).

High density EEG has been used to show re-mapping of motor representations in the brain closer to their original locations following targeted reinnervation in the upper limb (1, 39). Targeted reinnervation has mostly been studied for upper limb amputations and has been shown to be an effective method for producing sufficient EMG signals for the control complex multi-functional artificial arms (66). Because of the high prevalance of major lower limb amputations, more research on targeted reinnervation for the lower limb is needed. In 2012, a study was conducted to increase understanding of locations of sensory and motor nerves in transfemoral amputees (67). Targeted reinnervation is potentially an effective method for preventing neuroma pain after amputation (68, 69), along with improving sensation (70).

1.4 Myoelectric prostheses

1.4.1 Current state of the art

As of today, multiple different types of prostheses exist. Whether it’s an aesthetic prosthesis, a body-powered prosthesis or a myoelectric prosthesis, the choice of prosthesis should aim to suit each patient’s personal and future needs. A myoelectrically controlled prosthesis uses myoelectric EMG signals from a residual limb muscle to control movement. When the amputee intentionally engages a specific muscle, the prosthesis receives signals from the muscle and the data is used as command for the motor in the prosthesis (71). Most myoelectric prostheses have been designed for upper limb amputees and the current availability of myoelectric prostheses for lower limb amputees is scarce. Myoelectric prostheses are limited in regards to sensory feedback. Their action depends on visual observation while performing a motion or a hand grasp (72).

Össur has been researching sensory feedback and electromyogram (EMG) control for lower limb prosthetics and has investigated both non-invasive and invasive methods (73). As part of an Össur study, two amputees now have a year’s experience of controlling bionic prosthetic legs by using tiny implanted myoelectric sensors (IMES) (74). The IMES are surgically placed in residual muscle tissue and by using a receiver placed inside the socket, the IMES generates the desired movement of the prosthesis in real-time.

In upper limb research, sensory feedback systems have enabled feedback of sensations for the user and research suggests that sensory feedback systems have a positive effect on phantom limb pain (75). Össur has developed efficient sensors that are incorporated into their prosthetic limbs and the signals regarding position and load could be used as input for sensory feedback.

1.4.2 Effects of myoelectric prostheses on brain reorganization and phantom limb pain

Extensive and frequent use of myoelectric prosthetics is believed to have an effect on cortical reorganization and phantom limb pain. In a study of 14 unilateral upper limb amputees, fMRI was used to find that use of myoelectric prosthetics was negatively correlated with cortical reorganization and reduced phantom limb pain (76). It has been suggested that an EMG controlled prosthetic hand is recognized by the brain as a functioning alternative to a real hand through a mirror system in the brain (77). A systematic literature review found that the use of myoelectric prosthetics for the upper limb reduces phantom limb pain (78).

1.5 Phantom limb

Phantom limb is the sensation that a missing limb is still present and attached to the body. Phantom limb is often associated with pain and phantom limb pain can be severe. Phantom limb pain differs between person to person. In a study from 2013, on phantom limb pain after amputation, the pain was commonly described as a sharp or stabbing pain (47.4%), followed by a dull ache (34.0%), shooting/electric pain (33.0%), cramping (21.6%) and burning sensation (16.5%) (79). People who have undergone amputations often experience other sensations e.g. phantom sensations, that can be described as non-painful sensations and stump pain which is associated with the residual limb (80). Common non-painful sensations include tingling, itching and sensation of the missing limb (81).

1.5.1 Phantom limb pain prevalence after amputation

Even though results from studies vary, it is believed that most upper and lower limb amputees feel phantom limb sensations and pain at some stage (82-85). A study from 2011 reports that phantom limb pain is experienced by 60% to 80% of patients following amputation caused by trauma, injury or peripheral vascular diseases (80) and phantom limbs are believed to be experienced by up to 98 % of all patients immediately after amputation (86). In a study of major lower limb amputation in patients with peripheral vascular disease, the incidence of phantom limb pain was found to be 78.8%, and 51.2% had stump pain after a 6 months follow up period after amputation (87).

1.5.2 Mechanism of phantom limb pain

The current theories on phantom limb pain are based on peripheral and central neuronal mechanisms but overall, its pathophysiology is poorly understood and an agreement on the overall mechanism behind phantom limb pain is lacking (88). When an individual undergoes amputation, extensive changes in the damaged tissue occur. The peripheral aspect of phantom limb pain relates to the deafferentation of neurons and a formation of a neuroma in the stump, caused by regenerative growth of the severed axon (89). These neuromas exhibit spontaneous ectopic activity and increased sensitivity to chemical stimulus. Peripheral sensitization occurs which promotes primary hyperalgesia by minimizing the threshold for nociception activation (90).

The central aspect of phantom limb pain is twofold and refers to spinal plasticity as well as cortical reorganization (91). Following injury to peripheral nerves, a process called central sensitization occurs. The process leads to changes in the spinal dorsal horn, increased general excitability of neurons in the spinal cord, along with neuronal field expansion and down-regulation of inhibitory processes (92). Finally, cortical reorganization is believed to be an integral reason for phantom limb pain. Cortical reorganization in the somatosensory cortex explains why pain stimuli from adjacent areas to the missing limb might result in pain in the phantom limb. The amount of cortical reorganization in the somatosensory cortex has been positively associated with the intensity of phantom limb pain in upper limb amputees (93) and a reduction in cortical reorganization has been found to correlate with a reduction in phantom limb pain (94).

1.6 Research aim

This study aims to show for the first time how high-density EEG can be used to evaluate cortical reorganization and activity in the motor cortex in lower limb amputees. Research on cortical reorganization following lower limb amputation seems to be scarce. The aim is to localize cortical activity in the motor cortex generated by specific movement tasks and compare cortical activity in the healthy and amputated subjects. The objective is to gain further knowledge on the effect amputation has on the motor cortex of the brain.

2 Materials and methods

2.1 Participant selection

Medical doctors at Össur's headquarters in Iceland invited two participants, one healthy and one left transfemoral amputee to participate in the project. The inclusion criterion for the amputated individual was to be a transfemoral amputee. The exclusion criteria were being diagnosed with any neurological- or vascular disease and to be known to have adverse reactions from EEG prep gel. An inclusion criterion for the healthy participant was to be without an amputation. The exclusion criteria were the same as for the amputated participant.

In a meeting before the measurements took place, the following information was collected from the amputated participant: age, location of amputation, date of amputation, reason for amputation and use of prosthesis. The only information collected from the healthy participant was age and sex. The healthy participant was a 23-year-old man. The amputated participant, a 55-year-old man, underwent high transfemoral amputation of the left side of the body following a trauma in late 2011. Soon after amputation, the participant completed four weeks of mirror therapy that significantly reduced the experience of phantom limb pain. In early spring of 2012, the participant was fitted with Total Knee®, which is a polycentric knee provided by Össur. Later that year, he was equipped with Rheo Knee®, a microprocessor knee which he has been using since.

Both participants gave their written informed consent for the participation in the study, which was approved by The National Bioethics Committee in Iceland (VSN-15-151). The study was conducted in compliance with the principles of the Declaration of Helsinki.

2.2 Testing procedures

Testing was twofold; at first EEG and EMG data was collected while participants performed specific cued motor tasks in a relaxed, seated position. Secondly, EEG and EMG data was collected while participants walked for four minutes on a treadmill.

The healthy participant was instructed to perform a total of four motor tasks, one at a time, with first one side of the body and then the other in a seated position: knee extension and ankle dorsiflexion, while EEG and EMG signals were recorded. For the intact limb, the amputee was instructed to perform the same specific movements as the healthy participant while he was instructed to perform motor execution for the missing limb. Motor execution differs from motor imagery, where one simply imagines a movement without contracting any stump muscles (95, 96). Motor execution is an attempted movement that is perceived by the amputee as the phantom limb actually moves. These two processes show different EMG patterns recorded from the stump muscles. Motor execution provides EMG signals whereas motor imagery does not (97).

For each movement, two surface EMG electrodes were used to measure muscle activity. During knee extension, electrical activity from the rectus femoris and vastus medialis muscles was recorded. For ankle dorsiflexion, EMG signals from the tibialis anterior muscle were measured along with the rectus femoris, to ensure that the participants were not activating the knee extensors. The electrodes were placed according to SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) standards, which is a project in European recommendations for surface EMG placements and procedures. For the amputated limb of the amputee, the two electrodes were placed on the stump, one laterally and one medially. This helped to verify that the amputee was performing motor execution. The skin underneath each electrode was cleaned with Nuprep® skin preparation gel for improved signals. Participants were instructed to try to activate primary muscles used for each movement without other undesired muscle activation. Before collecting data, a training session for the participants was conducted. The amputee was asked to perform each movement on both limbs at the same time to provide a steady rhythm for the phantom limb during the training. In the training session, the movement rate was established and both participants felt comfortable moving the limbs as well as the phantom limb.

Each set of movements was performed thirty times and one movement was held for three seconds with a three second break in between. In order to prevent fatigue, longer, few minute breaks were in between each set of movements. The same movement rate was used for both intact limb and the phantom limb. Participants were asked to make consistent and strong movements and it was emphasized that participants should perform the movements with the same intensity and rate with both limbs. This was done to maintain the motor cortex signal levels from the intact limb and phantom limb at similar strength. Additionally, a screen provided visual information regarding when to execute the movement and when to relax while auditory beeps were presented to give the participants a pacing signal. A countdown timer was shown on the screen, counting from three to zero. At zero, a text appeared displaying motion commands e.g. right knee extension or right knee relax.

For the second part of the testing, participants walked on a treadmill at a comfortable pace, chosen by them while EEG data was collected. The healthy participant maintained a pace of 2.5 km/hour while the amputee maintained a pace of 1.6 km/hour, both for four minutes. For the healthy participant, EMG signals from both rectus femoris muscles were measured. For the amputee, EMG signals from the rectus femoris muscle of the intact limb and from the medial electrode location of the stump were obtained.

2.3 High-density EEG data collection and MRI

As part of a RANNÍS sponsored multi-institution project, involving Össur, Decode, Landspitali - National University Hospital of Iceland, University of Iceland, University of Reykjavik and Hjartavernd.

a 256-electrode high-density EEG system was recently acquired in Iceland. This high-density system was used in the study and EEG testing was done at the neurophysiology lab at Reykjavík University. This system allows for simultaneous EMG measurements in two channels. A 256-electrode cap was placed on the head of participants. Conductive gel was inserted into every electrode and impedance of each electrode was inspected before the start of the measurements to make sure that a good EEG signal could be acquired. After obtaining the EEG data, an infrared surgical camera was used to digitize the electrode locations. The digitized EEG electrode positions were used to combine EEG data with MR images.

MRI was performed at Hjartavernd. MR images were acquired on a single research-dedicated 1.5 T Signa Twinspeed EXCITE 16HDxt system (General Electric Medical Systems, Waukesha, WI) using a 8-channel phased array head cap coil. The structural image protocol consisted of a 1) T1-weighted three-dimensional fast spoiled gradient echo (3D-fSPGR) sequence (time to echo (TE), 2 ms; time repetition (TR), 7.8 ms; inversion time (TI), 400 ms; flip angle (FA), 12°; field of view (FOV), 240 mm; matrix, 256×256). Each volume consisted of 136 slices with 1.2 mm slice thickness and in-plane pixel size of 0.94 mm x 0.94 mm, 2) A spin echo – echo planar imaging (SE-EPI) based diffusion tensor imaging (DTI) sequence with 25 diffusion directions and 2.5mm isotropic voxels at 60 slice locations (TE, minimum full; ≈83 ms; TR, 16000 ms; FOV, 280 mm; matrix 128x128). The DTI images included three sets of T2*-weighted images (b=0) and 25 sets of diffusion weighted images (b=1000). All images were acquired to give full brain coverage.

The EEG and MRI data was imported into asa™, an EEG/ERP and MEG analysis software package. The MR images were used to construct three-dimensional images of the brain of each participant. The Standardized Low Resolution Electromagnetic Tomography (sLORETA) (98) method was used as the inverse solution for source localization of scalp potentials.

3 Results

3.1 Surface potential maps

The surface potentials, averaged over 30 movements, at a time interval of 3 seconds for each set of movements, were mapped over the cortex for each participant. The surface potentials and peaks in activity were recorded over time from different cerebral cortex locations. The following results were obtained from asa™.

3.1.1 Healthy participant

The following figure shows the average surface potentials acquired at specific moments when the healthy participant performed right knee extension. The left image displays the scalp potentials 200 milliseconds after commands for the right knee extension movement appeared on the screen. Here high activity in the visual cortex is observed, as well as considerable activity in the supplementary visual cortex, somatosensory association area and somatosensory cortex, more on the right side. The right image shows activity further ahead in time. At 334 milliseconds after visual command we observe activity in the prefrontal cortex, supplementary motor area and pre-motor cortex. Maximal activity in the motor cortex was observed along the longitudinal fissure.

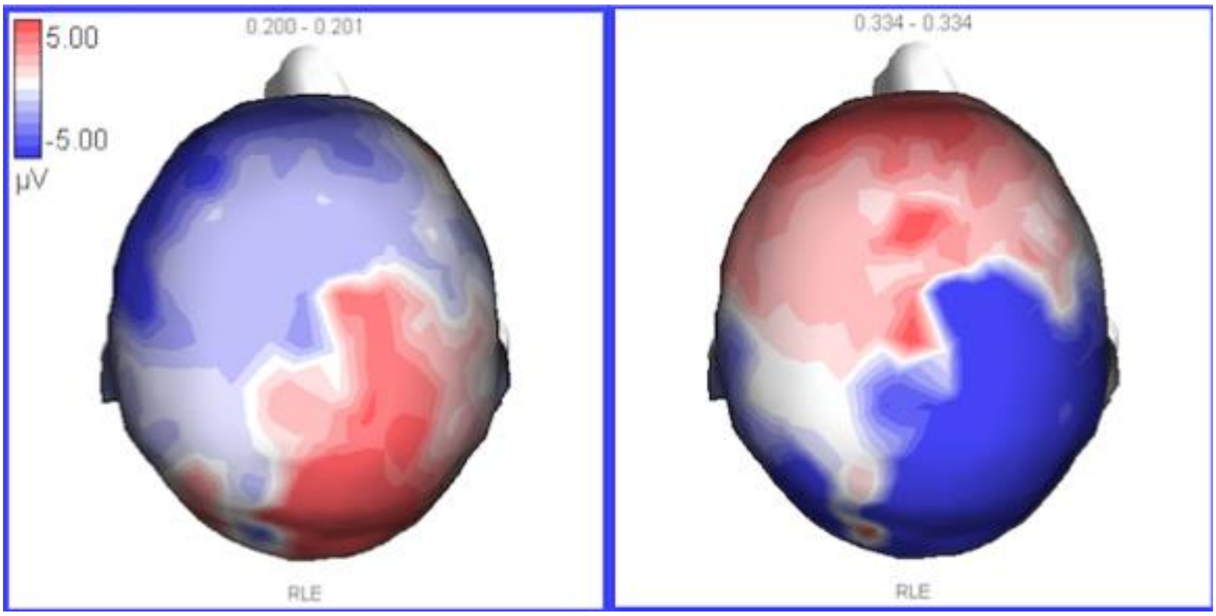

Figure 1 Surface potential mappings for right knee extension for the healthy participant. The scale represents voltage [µV] while numbers represent time [s]

3.1.2 Amputated participant

Figure 2 shows cortical activity 118 and 207 milliseconds after visual commands for left knee extension of the amputated knee were displayed on the screen respectively. The left image shows maximal activity recorded in the visual cortex while also high activity in the prefrontal cortex and motor cortex. The image on the right shows high motor cortex activity along the longitudinal fissure but also activation more laterally in the area of the primary motor cortex.

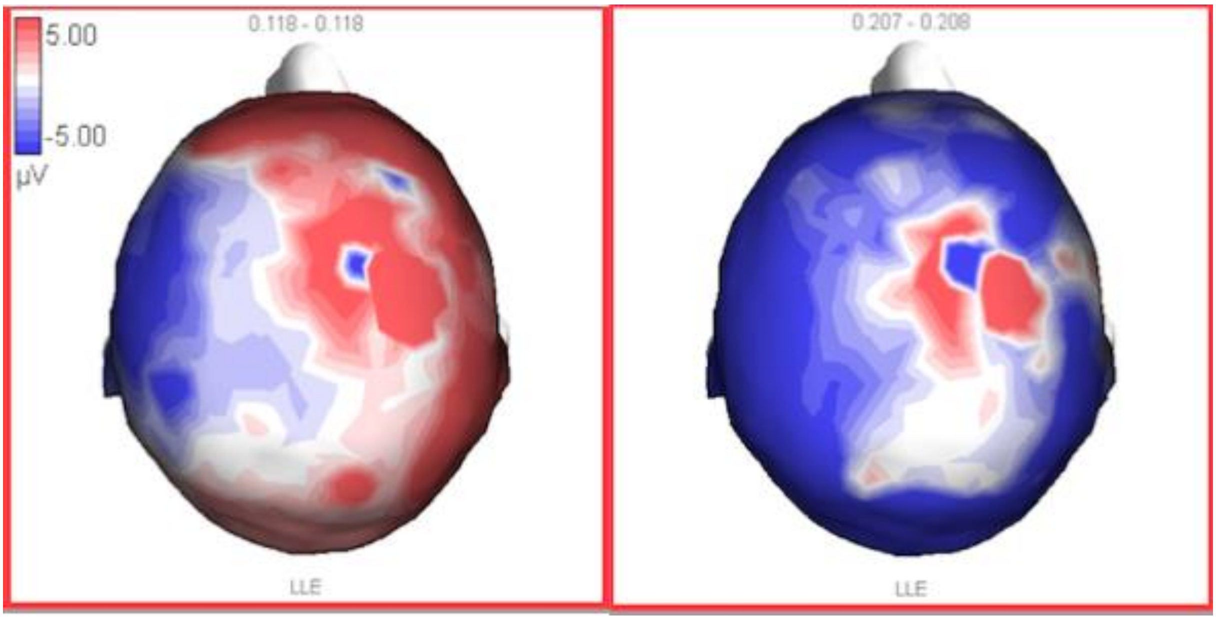

Figure 2 Surface potential mappings for attempted extension of the amputated left knee. The scale represents voltage [μV] while numbers represent time [s]

3.2 Potentials within the cortex

We located activity generated from within the cortex. The processing of signals within the cortex is still in early stages. Figure 3 shows the maximum activity recorded while the healthy participant executed right knee extension. It shows high amount of activity in the parietal lobe as well as activity in the occipital cortex. The left image is in sagittal plane while the right image is in axial plane.

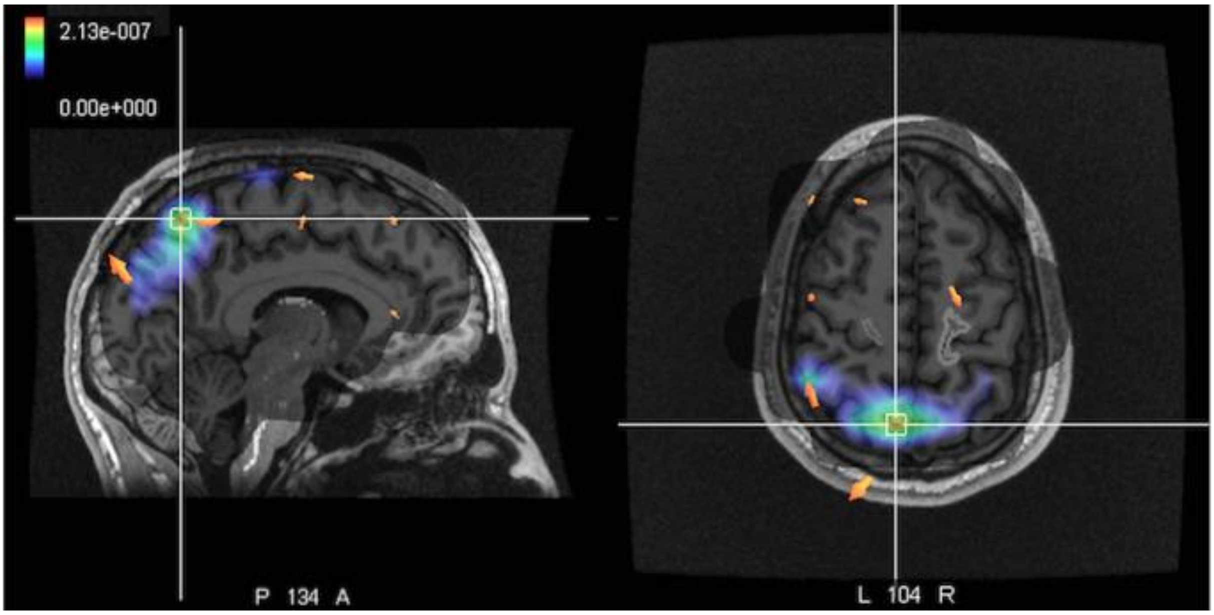

Figure 3 Potentials within the cortex for the healthy participant - right knee extension. The scale represents voltage [V]

3.3 Diffusion tensor neural tract imaging

Figure 4 shows the preliminary DTI neural tract results. An MSc student at the University of Reykjavík is currently working on processing the DTI data. Here, the corticospinal tracts are observed in coronal plane. Red and yellow show the corticospinal tracts while the green and blue show hippocampal tracts.

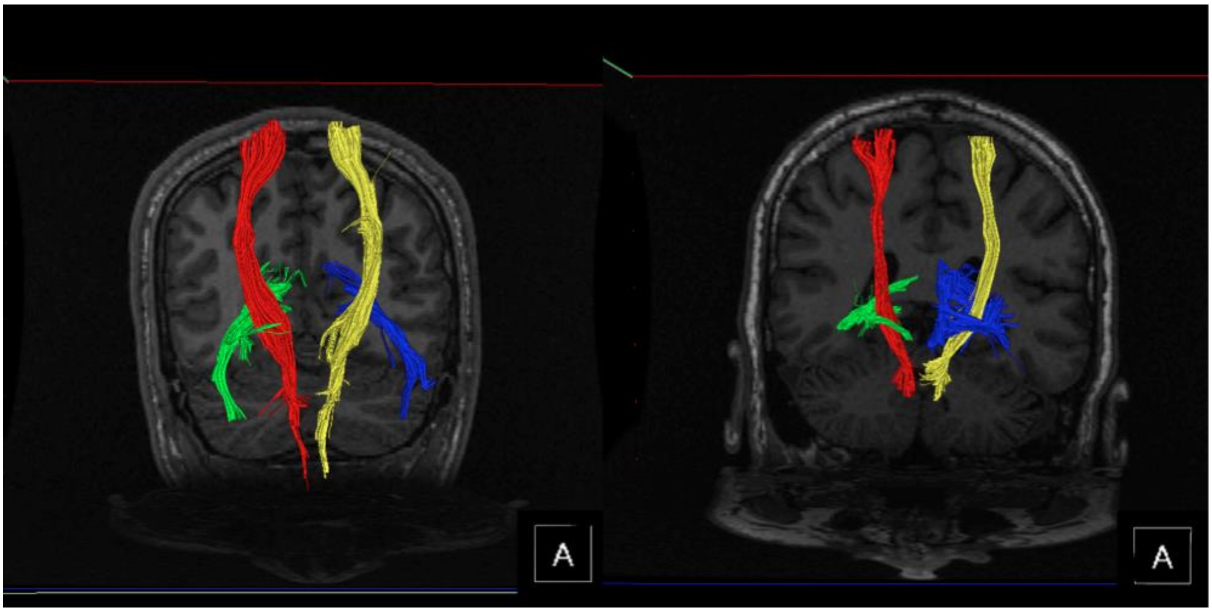

Figure 4 DT image results showing neural tracts within the brain

4 Discussion

By using high-density EEG we have managed to observe cortical activity in different parts of the brain at different points in time. Preliminary results from the amputated participant suggest the presence of cortical reorganization in the motor cortex. When the amputee tried to voluntarily extend the left amputated knee, high cortical activity was observed in the motor cortex near the longitudional fissure but a large activation was also observed more laterally which may point to cortical reorganization. According to the topography of the motor cortical homunculus, the area which activated more laterally should correspond to trunk, neck, head or upper limb, but not lower limb movement. To our knowledge this study was the first to use high-density EEG to map out cortical reorganization in the motor cortex following lower limb amputation. The findings might provide support for the effectiviness of high-density EEG to observe cortical reorganization following lower limb amputation. The amputated participant did not express any discomfort nor fatigue during the EEG measurements, which is encouraging for future measurements of more participants.

We realize that the activation we observe in this area could simply be the result of other muscle activity, e.g. muscle activity associated with maintaining body posture. Currently, we can only measure muscle activity using two EMG electrodes. More EMG electrodes would be needed to be able to monitor other muscle activation that could interfere with the results. More comprehensive data analysis is needed for better source localization related to each set of movements and there might be a need for acquiring new EEG processing software. Furthermore, more analysis for the DTI neural tract data is needed. It would be interesting to investigate if structural white matter changes, associated with the amputated side, could be observed for the amputated participant. Data processing for the treadmill EEG and EMG data is remaining as well. A lot of noise is expected due to the abundance of muscle activity generated during walking.

The development of myoelectric lower limb prostheses, as well as research into ways to pass sensory information from the prostheses to the user, has been going on in Iceland in recent years. The objective is to try to assess the impact of the use of such sensory feedback technology and myoelectric prostheses by examining the cortical reorganization in the motor and sensory cortices. Another objective is to examine the relationship between cortical reorganization, myoelectric prostheses and phantom limb pain.

The number of amputations in the USA is estimated to double by the year 2050 (8) and we might also expect similar increase in Iceland. In light of that development, there is a clear necessity for further research and development of better, more intuitive prostheses for upper and lower limb. Rehabilitation should aim to increase quality of life for amputees and designing more suitable prosthetics that give amputees better physical control is fundamental to that mission. The reversal of cortical reorganization towards a more natural cortical organization might be helpful for users to control a myoelectric prosthesis along with reducing phantom limb pain. The development of a technique to give amputees sensation of their prostheses should be essential in the design of future myoelectric and other prostheses. Targeted reinnervation has been successfully performed for upper limb amputees and enables them to control their prostheses while regaining sensory feedback. Although an exciting technique, it would seem that in some cases a less invasive method, that would not involve the general risks of surgery, paralysis of the target muscle or the recurrence of phantom limb pain (99) would be more appropriate.

These results encourage more research and indicate that cortical reorganization and motor cortical activity following lower limb amputation can be observed using high-density EEG. That being said, we want to be careful in drawing conclusions, as this is a pilot study involving only two participants. The results from this study could provide valuable information for further research on cortical reorganization planned in Iceland. The use of high-density EEG could be further developed to assess long-term reorganization of motor representations and the potential return of more normal cortical expression with EMG controlled prosthetic lower limb use. The method could be used to evaluate the effects of providing neurological control and sensory feedback to lower limb prosthetic users. This is in line with a study on neural interfaces implanted in amputees, where it was recommended that the brain's neuroplasticity should be monitored before, during, and after the implant (100)

This project was recently awarded with a science fund grant from Landspitali, The National University Hospital of Iceland, for further research, measuring and data analysis. The study is approved for a total of 10 participants. Research is ongoing but preliminary results are encouraging. Using EEG could be a practical, non-invasive method for monitoring rehabilitation of lower limb amputees and assessing prosthetic device performance and could have a future effect on rehabilitation protocols, not only in Iceland, but worldwide.

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