

RESTING-STATE EEG ANALYSIS IN CHRONIC STROKE PATIENTS

Comparing power spectra between hemispheres and between subjects as complementary method to the BSI and DAR



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Author:
Mique Saes, BSc.

Supervisors:

Prof. Dr. G. Kwakkel
Dr. E.E.H. van Wegen
C. Winters, MSc.
Prof. Dr. A. Daffertshofer

Summary

Background and objectives. Prediction of functional outcome post stroke has been a focus for several years. Electroencephalography (EEG) can be used to determine parameters such as the Brain Symmetry Index (BSI) and Delta Alpha Ratio (DAR). These parameters may improve prediction of functional recovery of the upper extremity post stroke. Most studies focused on the acute phase. Therefore, changes in EEG during recovery are poorly investigated. The first aim of the present study was to investigate whether the BSI and DAR differ between chronic stroke patients and healthy individuals. The second aim was to investigate whether comparing normalized power spectra over a range from 1 to 49Hz between hemispheres and between subjects could be complementary to respectively the BSI and DAR.

Methods. 64-channel EEG data was acquired from 8 young healthy individuals, 4 chronic stroke patients (≥ 6 months post stroke) and 4 healthy roughly age-matched older adults. Asymmetry in brain activation was investigated by calculating the BSI and comparing the normalized power spectra between the hemispheres. Differences in relative spectral power were investigated by calculating the DAR and comparing the normalized power spectra between the groups. Power spectra comparisons were conducted using a wavelet-based functional ANOVA (wfANOVA).

Results. A Kruskal-Wallis test revealed that the BSI differed between the groups ($\chi^2(2) = 8.487$, $P < 0.05$). Post-hoc analyses with Bonferroni correction revealed that the BSI of chronic stroke patients (Mdn=0.21, IQR=0.018) is significantly higher compared to the young (Mdn=0.11, IQR=0.026) and older (Mdn=0.11, IQR=0.004) controls ($P < 0.001$). A wfANOVA revealed no differences between the normalized power spectra of the hemispheres in healthy individuals. By contrast, all chronic stroke patients showed significant differences in relative power in the alpha and theta band. A Kruskal-Wallis test showed that the DAR did not differ between the groups ($\chi^2(2) = 3.429$, $P = 0.180$). Also the wfANOVA showed no differences between the normalized power spectra of the groups.

Conclusions. It was concluded that in the current study it was possible to discriminate chronic stroke patients from healthy individuals based on the BSI, but not based on the DAR. With regard to both parameters, a wfANOVA method is concluded to be complementary as it provides insight into the differences in relative spectral power between the hemispheres and groups. A BSI based on the theta band only, as well as a DAR based on the lesioned hemisphere may augment the discrimination between chronic stroke patients and healthy controls, while a BSI based on alpha activity may not. The BSI and DAR are both suggested to be investigated in a longitudinal study, to evaluate their development during recovery and their capacity to predict functional recovery.

Keywords: Stroke; EEG; Brain Symmetry Index; Delta Alpha Ratio; wavelet based functional ANOVA.

Abbreviations: EEG = Electroencephalography; BSI = Brain Symmetry Index; DAR = Delta Alpha Ratio; wfANOVA = wavelet-based functional ANOVA; ARAT= Action Research Arm Test; FMA = Brunnstrom Fugl-Meyer Assessment.

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1 Introduction

Worldwide 15 million people suffer a stroke each year. Stroke comes with a high mortality rate (approximately 18%) and is considered to be the second leading cause of mortality (Towfighi and Saver, 2011). Besides, it is the most common cause of severe long-term disability (Adamson et al., 2004). Approximately 80% of the stroke survivors suffer from a paresis of the upper extremity immediately after stroke onset (Nakayama et al., 1994). Unfortunately, only about 30% of the survivors will regain some dexterity six months post stroke (Wade et al., 1983; Sunderland 1994; Dobkin 2005; Nijland et al., 2010). In addition, the majority of patients will continue to experience functional limitations of the upper limb in daily living (Wolf et al., 2006). Being able to use the upper extremities is important in completing basic activities of daily living such as feeding, dressing, toileting and bathing independently (Veerbeek et al., 2011). In addition, also during translational movements the upper limbs are used (Snoek et al., 2004). Therefore, impairments of the upper extremities are associated with diminished health related quality of life (Nichols-Larsen et al., 2005). If present, the functional motor recovery after stroke is complex and may involve two complementary recovery processes referred to as restitution and substitution. Restitution assumes that improvements in function are due to 'true' neurological repair, in other words recovery of neural pathways that were damaged due to stroke. Substitution includes adaptive processes like compensation and reorganization (Rothi and Horner, 1983; Van Kordelaar et al., 2012; Kwakkel et al., 2004).

The time course of these complementary processes and the prediction of functional recovery of the upper extremity after stroke are crucial for general understanding of recovery post stroke and are of major interest for stroke survivors and caregivers. This has been a focus of rehabilitation researchers for several years.

Early prognosis of functional recovery after stroke may give clinicians additional information about the recovery progress. This could help to decide which therapy may be more useful and will help to optimize treatment goals. For example, if some return of dexterity is predicted, intervention should be focused on improving function of the paretic arm, while in case of a poor prognosis clinicians may teach patients how to deal with their deficits and allow compensation strategies (Kwakkel et al., 2003; Stinear et al., 2012). In this way therapy can be personalized and patients can be better informed about their recovery. Nowadays, the prediction of functional recovery of the upper extremity after stroke is based on clinical scores (Nijland et al., 2010). Patients able to perform some finger extension and shoulder abduction on the second day post stroke, have a probability of 98% to regain some dexterity. In contrast, patients who were not able to perform these movements had a probability of 25% to regain some dexterity (Nijland et al., 2010). Besides a sensitivity of 0.89, a specificity of 0.83 and a positive predictive value of 0.93, the Two-way contingency table analysis performed in this study showed a negative predictive value (NPV) of 0.76. As the NPV reflects the part of patients who did not regain some dexterity as predicted, it indicates a large number of false negatives (Nijland et al., 2010). Therefore, prediction is not yet optimal and can possibly be improved by taking into account neurophysiological parameters.

Besides functional tests, accurate prediction of outcome may be improved by neurophysiological parameters that reflect the amount of brain damage. Recovery of function after stroke is largely due to reorganization of activity in (sub)cortical networks (Carter et al., 2012a). A fMRI study revealed a temporal relationship between recovery and task-related activation of the motor system after stroke (Ward et al., 2003). However, one of the major disadvantages of fMRI is the poor temporal resolution. Activity of the brain can also be measured using scalp recorded electroencephalography (EEG), which is used as a direct measurement of local field potentials on a millisecond time scale. These potentials are mainly generated by the summed postsynaptic electric current of multiple neurons at the cortex (Teplan, 2002). In this way neurophysiological changes in the brain due to stroke may be reflected in the EEG. Stroke may for example change the absolute and relative spectral power of different frequency bands, resulting in an altered spectral power distribution. Therefore, EEG-based parameters can reflect neurophysiological changes.

The current study is part of the 4D-EEG project, which consists of a cross-sectional and a longitudinal study. In the longitudinal study the EEG of acute stroke patients will be measured during the recovery period from the subacute phase to six months post stroke. Measurements will take place at week 1, 5, 12 and 26 post stroke. From these EEG recordings, the changes of EEG-based parameters over time will be investigated and correlated with the amount of recovery of arm and hand function. Subsequently, it can be investigated if these parameters may serve as predictors of functional recovery of arm and hand function, and whether these EEG-based parameters could improve the prediction of functional recovery of the upper extremity. Prior to the longitudinal 4D-EEG study, a valid and reliable method should be identified to determine which EEG parameters may have a predictive value concerning functional recovery post stroke. These

parameters may be identified in the cross-sectional 4D-EEG study. The current study is based on part of the cross-sectional data.

Previous studies suggest that changes in EEG due to stroke relate to functional impairments. Reorganization of (sub)cortical networks is not only visible when networks are active, but also in resting-state. For this reason, resting-state activity may relate to behavioral deficits (Carter et al., 2012a). Two resting-state EEG-based parameters which have been suggested to be related to functional recovery in acute stroke patients are the Brain Symmetry Index (BSI) (Van Putten et al., 2004; Van Putten, 2007; De Vos et al., 2008) and the Delta Alpha Ratio (DAR) (Finnigan et al., 2007; Claassen et al., 2004).

Brain Symmetry Index is defined as a particular asymmetry in spectral power between the two cerebral hemispheres (Van Putten et al., 2004). It is assumed to reflect the amount of activity imbalance between the hemispheres, which is a common finding in stroke patients (Stojanovic and DJurasic, 2013). In this way, the asymmetry caused by stroke leads to higher BSI scores in acute stroke patients compared to healthy controls (Van Putten and Tavy, 2004). The BSI has been associated with the unilateral paresis of the upper limb, caused by a unilateral lesion in the vicinity of the central sulcus of the contralateral hemisphere. Enhanced activity of the penumbra has been suggested to be one of the factors that contributes to recovery of upper limb function (Zhang et al., 2013). Recovery of the penumbral tissue may result in a decreased asymmetry reflected by a decreased BSI. Moreover, BSI scores correlate with the clinical neurological condition of acute stroke patients quantified using the National Institutes of Health Stroke Scale (NIHSS) (Van Putten and Tavy, 2004). For this reason, the BSI may be an eligible prediction parameter for functional recovery in longitudinal rehabilitation studies. However, the development of the Brain Symmetry Index during recovery post stroke has not been investigated, as most studies focus on the (sub)acute phase.

Stroke may also result in changes of the average spectral power in different frequency bands. Activity in the alpha frequency band has been assumed to play a key role in optimal functioning (Bazanov, 2012). In stroke patients, who suffer impaired control, indeed a decreased resting-state alpha power was observed (Finnigan and Van Putten, 2013; Nuwer et al., 1987). Besides, Bazanova (2012) showed that alpha power increases from young age until puberty after which it is constant until the age of forty when it starts to decrease. In addition, an increased spectral power in the *delta* frequency band is commonly observed in stroke patients during resting-state (Finnigan et al., 2004; Finnigan and Van Putten, 2013; Nuwer et al., 1987). John and Prichep (2006) suggested that this originates in the deep cortical layers and the thalamus, and may be a reflection of inhibition of cortical neurons, resulting from dedifferentiation of neural activity. Another study found that cortical gray matter lesions alone do not lead to increased delta activity. They suggested that this type of lesions destroy the neuronal generators in the cortex (Gloor et al., 1977). They also found that that lesions of subcortical white matter does lead to increased delta activity. Therefore, they suggested that the delta activity could be the result of cortical deafferentation and originates from the cortex overlying the lesion (Gloor et al., 1977). These suggestions are supported by the finding that delta activity is often increased at the location of the primary injury and deafferented regions. Structural damage due to stroke results in increased delta activity and decreased alpha activity. The relation between delta and alpha power is referred to as the DAR [Delta Alpha Ratio] (Finnigan and Van Putten, 2013; Nuwer et al., 1987). A higher value of the DAR has been associated with poor functional recovery, while preserved background alpha activity and absence of slow activity was found in patients with good functional recovery (Finnigan et al., 2007; Burghaus et al., 2007). Besides, an animal study showed that the DAR was increased in rats three hours after stroke onset, but returned to almost normal values in the chronic phase. In addition, higher DAR values correlated with decreased motor function of these rats (Zhang et al., 2013). Therefore, the DAR may be suitable as a biomarker of functional recovery in longitudinal rehabilitation studies. However, in humans the development of the Delta Alpha ratio during recovery post stroke has not been investigated.

For both parameters, BSI and DAR, a wavelet-based functional ANOVA (wfANOVA) in which resting-state power spectra are compared could be a complementary method. The BSI is averaged over a frequency range from 1 to 25Hz, which will presumably enhance robustness and reliability (Van Putten, 2007). However, this also results in loss of information concerning the specific frequency bands at which the asymmetry of stroke patients may be increased. Moreover, the BSI may also be averaged over frequencies at which the asymmetry did not deviate from healthy individuals. This may impede discrimination between stroke patients and healthy individuals. One option of improvement is by calculating the BSI for each of the 25 frequencies and investigate at which frequencies the BSI differs between stroke patients and healthy individuals. However, this will induce the multiple comparisons problem. Another method to investigate asymmetry of brain activation is by comparing normalized power spectra between the hemispheres. This method can be complementary as it may provide insight into the frequency at which the relative power differs

between the hemispheres. However, when comparing power spectra at each separate frequency (i.e. comparison in the frequency domain) the power of the outcomes should be corrected for the number of comparisons. This will result in a multiple comparisons problem as well. As a power spectrum is a fast converging function, it can be expressed in a low number of wavelets. So, if a power spectrum is transformed from the frequency to the wavelet domain, the number of necessary comparisons will be decreased. Therefore, this will avoid the multiple comparisons problem. A method like this has been developed and is called the wfANOVA (McKay et al., 2013). The wfANOVA might be complementary to the asymmetry analysis based on the BSI, as it provides insight into the frequency at which the relative power differs between the hemispheres.

Comparing the power spectra of stroke patients and healthy individuals may also be complementary to the DAR. It can provide insight into the anomalies at a wider frequency range than just the delta and alpha band. Using the wfANOVA to compare power spectra of stroke patients and healthy individuals, it can be investigated at which frequencies the relative spectral power is affected by stroke and how this relates to the DAR.

Previous research, in which the EEG data of stroke patients was recorded several times between 3 weeks and 6 months post stroke, showed that the mean relative delta and theta power were increased at the lesioned hemisphere compared to the non-lesioned hemisphere, while the relative alpha power was decreased. At the lesioned hemisphere, the relative delta power decreased over time, while the relative theta and alpha power increased (Giaquinto et al., 1994).

However, it is still unclear how the power spectra of stroke patients differ from healthy individuals, and whether they return to normal during the recovery period.

The first aim of the current study was to investigate whether the BSI and DAR differ between chronic stroke patients and healthy individuals, which information is lacking in the current literature. The second aim was to investigate if a wfANOVA may be complementary to the BSI and DAR, and whether it is possible to refine the calculation of these parameters. By applying a wfANOVA it could also be investigated at which frequencies the relative spectral power of chronic stroke patients differ between the hemispheres, and how their power spectra differ from those of healthy individuals. In addition, the influence of age was examined.

In the acute phase the BSI will be increased due to stroke, where after it will decrease during recovery. However, there will always be a part of the brain, which is irreversibly damaged. Therefore, it is hypothesized that the BSI of stroke patients in the chronic phase is higher compared to that of healthy individuals.

The power of the delta activity associated with brain damage is assumed to be increased in the acute phase post stroke while the power of alpha activity reflecting healthy brain activity is hypothesized to be decreased due to stroke. During recovery these values are expected to change in the direction of normal values. Therefore, the DAR of chronic stroke patients is hypothesized to be slightly higher compared to healthy individuals, depending on the amount of recovery that took place.

The analysis based on wfANOVA complements the current analyses of asymmetry, because it may provide insight into the difference in relative spectral power between the hemispheres per frequency instead of an asymmetry based on the average over a range of frequencies. It is hypothesized that in chronic stroke patients this asymmetry will be present at the delta, theta and alpha frequency band. The wfANOVA will also be complementary to the common analysis of the DAR, since the comparison of power spectra between chronic stroke patients and healthy individuals provides insight into differences of the relative contribution of each frequency band instead of just the ratio between the spectral power of the delta and alpha band.

In this study the influence of age on asymmetry and spectral power was studied by comparing the resting-state EEG of young healthy individuals and healthy elderly. To the best of our knowledge there is no reason to expect differences in asymmetry due to age. In contrast, the spectral power at the alpha frequency band is hypothesized to be lower in elderly compared to young individuals, resulting in a higher DAR.

2 Methods & Procedures

2.1 Subjects

The present study is part of a large cohort EEG study (European Research Council, 4D-EEG project). The study has been approved by the Medical Ethical Reviewing Committee of the VU University Medical Center Amsterdam (registration number 2014.140). The analyses are based on a part of the cross-sectional data as the measurements are not yet finished. Two groups of healthy participants without a history of neurologic disorders were recruited. The mean age of these groups differed in order to be able to investigate the influence of age. These groups are defined as the young-group and elderly-group. Furthermore, four stroke patients were included to compare resting-state EEG between chronic stroke patients and healthy controls. The inclusion criteria of these patients were: 1) a first-ever ischemic or hemorrhage cerebral stroke; 2) ≥ 6 months post stroke at which no further recovery is expected; 3) functional upper limb impairment in the acute phase; 4) ≥ 18 years of age; 5) mini mental state examination score ≥ 20 and 6) written informed consent. Exclusion criteria were: 1) a pacemaker or other metallic implants; 2) upper extremity orthopedic limitations; 3) previous or recurrent stroke.

2.2 Procedures

The participants signed a written informed consent after which the EEG measurement started. To prevent drowsiness during the resting-state EEG measurement, the measurement was divided in five trials of one minute. During the measurement the participant was seated in a wheelchair with eyes opened and was asked to focus on a spot just below eye level that was presented on a computer screen. Several healthy individuals were measured twice (test-retest design). Furthermore, upper limb function of the chronic stroke patients was assessed with the upper limb subtest of the Brunnstrom Fugl-Meyer Assessment (FMA) and the Action Research Arm Test (ARAT), respectively reflecting the amount of motor function and upper limb capacity as measures of functional recovery (Fugl-Meyer et al., 1975; Lyle, 1981).

2.3 Data acquisition

Electroencephalography

The EEG measurements were conducted in a specially equipped van. A high-density 64-channel EEG recording was performed using Ag/AgCl electrodes and a 64-channel amplifier (Refa64, TMSi, Oldenzaal, the Netherlands). Recordings were performed at a rate of 2048Hz using ASA software (ANT software BV, The Netherlands). The ground electrode was placed on the mastoid process. During the measurements the data was average referenced. To avoid polarization the electrode impedance was kept below 20k Ω .

Clinical tests

Two valid and reliable clinical tests to measure the upper limb capacity and motor function are respectively the Action Research Arm Test (ARAT) and the Fugl-Meyer Assessment (FMA) (Van der Lee et al., 2001; Duncan et al., 1983). The FMA is an impairment scale which is specific for stroke patients and determines the ability to execute dissociated movements. It is a good predictor of upper extremity motor recovery and is most likely to reflect true neurological motor recovery (Levin et al., 2009), in the 'body structure and function' domain of the International Classification of Functioning, Disability and Health (ICF) model. To test the motor function of the upper limb only the upper extremity domain of the FMA will be assessed. In this test a maximum score of 66 can be achieved, in which a higher score corresponds to better motor function. The ARAT is an arm-hand test which focuses on manipulating objects and correlates strongly with other measures of upper-extremity function (Lang et al., 2013) in the 'activity' domain of the ICF model. It consists of 19 items covering grasp, grip, pinch or gross movements. Each task in the ARAT is scored on a four-point ordinal scale (0-3) in which a higher score represents better function, resulting in a maximum score of 57.

2.4 Data analysis

To investigate whether the DAR and BSI differ between chronic stroke patients and healthy individuals, the outcomes of the different groups were compared. These statistical tests were performed using IBM SPSS Statistics version 20 (IBM, Armonk, NY, USA). Subsequently, the power spectra were compared between hemispheres and between groups by applying the wfANOVA using MatLab R2014a (The MathWorks Inc., Natick, United States). Thereafter, outcomes of the BSI and DAR were compared to the outcomes of the analyses based on the wfANOVA. In addition, it was tested whether age influences the analyses. Furthermore, a new BSI was calculated based on the asymmetry at the frequency band at which the spectral power differed significant between the hemispheres in accordance to the wfANOVA. In addition, refinement of the DAR was investigated.

2.4.1 Preprocessing

EEG data was filtered offline with a 0.5Hz 4th order high pass Butterworth filter using MatLab. Data preprocessing was conducted using the FieldTrip toolbox for EEG/MEG-analysis (Oostenveld et al., 2010). Band-stop filters with ranges of 49-51Hz, 99-101Hz and 149-151Hz were used to reject 50Hz line-noise. This noise was caused by the necessary connection of the van to the AC power during the measurement. The artifact rejection method consisted of the exclusion of eye-blinking and eye-movements using independent component analysis (ICA). If necessary, electrode recordings were excluded from calculations comparable to ignoring bad channels, where after the data was average referenced again. The power of scalp EEG varies between individuals due to among others anatomical characteristics. Therefore, it is necessary to normalize the power spectra in order to be able to compare them between individuals. This normalization was performed by dividing the power by the integral of the power spectrum from 1 to 49Hz, for each channel separately.

2.4.2 BSI calculation

The BSI is defined as the average of the absolute value of the pair-wise mean difference in spectral power between the left (*L*) and right (*R*) hemisphere in the frequency range from 1 to 25 Hz (Adapted from Van Putten and Tavy, 2004). That is, per channel-pair *c* one computes

$$BSI_c = \frac{1}{25} \sum_{f=1}^{25} \left| \frac{R_c(f) - L_c(f)}{R_c(f) + L_c(f)} \right| \quad (1)$$

which is followed by averaging over all channel pairs.

$$BSI = \frac{1}{c} \sum_{c=1}^c BSI_c \quad (2)$$

The upper bound of the BSI is one, reflecting maximal asymmetry for all channel-pairs. The lower bound of the BSI is zero, representing perfect symmetry. The BSI of healthy individuals is assumed to differ from zero due to activity in the default mode network, which is most active in resting-state. This network is not equally distributed over the two hemispheres, resulting in lateralisation at which no pathology will be expected (Swanson et al. 2011).

In the present study, power spectral density was determined using the MatLab function *ft_freqanalysis* of the FieldTrip Toolbox using Hanning windows in a multi-tapering approach. The artifact-free resting-state EEG data of all electrodes was analyzed, excluding the electrodes of the Z-line. In addition, whenever one of the electrodes of a channel-pair was missing, the corresponding channel-pair was excluded.

To investigate whether the asymmetry differs between chronic stroke patients and healthy individuals, the BSI was calculated for each individual after which it was investigated using a non-parametric one-way between subjects ANOVA (Kruskal-Wallis Test), followed by a post hoc test using Bonferroni corrections. A non-parametric test was applied as the sample sizes of the groups were unequal. To be able to examine the influence of age, the healthy individuals were divided in two groups; young and older individuals.

2.4.3 Wavelet based functional ANOVA

McKay et al. (2013) developed the wfANOVA in order to compare electromyography (EMG), signals that vary as a function of time, to overcome the multiple comparisons problem. In the current study asymmetry was analyzed by comparing the power spectra between the hemispheres. When the power spectra are compared in the frequency domain, an ANOVA should be performed for each frequency of the power spectra, where after it is necessary to correct for the large number of performed tests. This results in a major decrease of statistical power, which reduces the chance to find a significant difference. Power spectra of EEG data very quickly reach an asymptotic form and can therefore be described by just a few wavelets.

In the wfANOVA function the power spectra were first transformed to the wavelet domain (Figure 1A). This transformation function uses a discrete-wavelet-transform (*dwt.m*, *Wavelet Toolbox Matlab*) and 3rd order coiflet-type wavelets (Figure 2). This type of wavelets is nearly-symmetric. In addition, coiflets have been shown to be excellent when approximating the sampling of a smooth function (Wei et al. 1997). This wavelet transformation provides a set of wavelets for each of the power spectra. Subsequently, the wavelets describing the power spectra of the left hemisphere were compared with those of the right hemisphere using a repeated-measures ANOVA followed by pairwise comparisons with Bonferroni correction. Due to the expected reduction of necessary comparisons, this can result in a higher statistical power, which increases the chance of finding a significant difference. If there is a significantly different wavelet, this wavelet was inverse transformed from the wavelet domain to the frequency domain (Figure 1B). Subsequently it was

plotted in order to show at which frequency the power spectra differ between the left and right hemisphere.

To examine the asymmetry within an individual using the wfANOVA, the power spectra of the electrodes of the left hemisphere were compared to the power spectra of the right side, in which the electrodes were paired (Appendix A). Therefore, the power spectrum of the artifact-free EEG data was calculated for each channel using the *pwelch* function of MatLab. The window was set on five times the sampling frequency, with an overlap of 50% and a sampling frequency of 2048Hz. Subsequently, each power spectrum was normalized by dividing it by the sum of its spectral power over a range of 1 to 49Hz. These normalized power spectra served as the input of the repeated-measures wfANOVA.

It was first tested whether and at which frequency the power spectra of the left electrodes were different from those of the right electrodes for each of the healthy individuals separately. Subsequently, the asymmetry of each of the 4 chronic stroke patients was investigated the same way. This provides information concerning the frequency band at which the relative contribution to the total power differed significantly between the hemispheres. Thereafter, the outcomes of the three groups were compared to identify influences of age and stroke.

To see whether the wfANOVA is complementary to the BSI, the outcomes of both methods were compared to see whether the results were congruent. Besides, the BSI was recalculated based on the spectral power of the frequency band at which the difference of spectral power between the hemispheres was significant. To investigate whether this resulted in augmented discrimination between chronic stroke patients and healthy individuals, this recalculated BSI was compared between the groups using a non-parametric one-way ANOVA (Kruskal-Wallis Test).

Consistency and reliability of the BSI and asymmetry based on power spectra

To test whether a method based on wfANOVA is consistent during a measurement, the five resting-state trials of each subject were analyzed separately after which the outcomes of the consecutive trials were compared. The same was done for the BSI, after which per group a Friedman Test was applied. A non-parametric test was applied as the sample sizes are very low. If this test reveals significant difference, Wilcoxon Signed Rank Tests with Bonferroni correction were performed as Post-Hoc tests.

The reliability of both methods was tested using a test-retest design. Resting-state EEG of six healthy subjects was measured at two different days with a time interval between two and seven days. Reliability of the BSI was tested using a Wilcoxon signed rank test. Reliability of asymmetry based on wfANOVA was evaluated by comparing the outcomes of the test and retest for each subject.

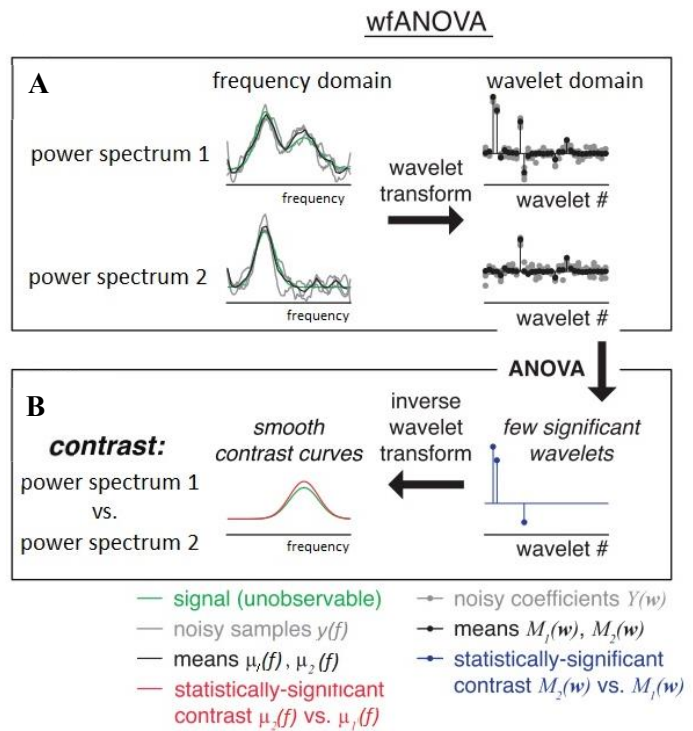


Figure 1 Visualization of the comparison of power spectra using a wfANOVA. **A.** Two datasets of spectral power are transformed from the frequency domain to the wavelet domain. Subsequently, these wavelets are compared using an ANOVA with Bonferroni correction. **B.** The wavelets that are significantly different between the two datasets will be inverse transformed to the frequency domain, to visualize the difference between the power spectra. Adapted from McKay et al. (2013) *Journal of Neurophysiology*.

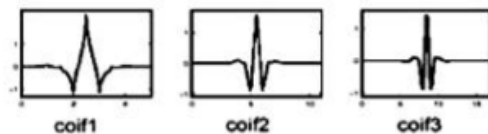


Figure 2 Visualisation of the different orders of the coiflet-type wavelets. In this study a 3rd order coiflet-type wavelet was used. Adapted from Bustillos and Pirela (2013) *Revista de la Facultad de Ingenieria U.C.V.*

2.4.4 DAR calculation

The DAR is defined as the ratio of the delta power to the alpha power. For each channel (c) the power of the delta frequency band (D_c) was calculated as the sum of the spectral power ($P_c(f)$) over a frequency range from 1 to 4 Hz (3). In the same way the power of the alpha frequency band (A_c) was calculated over a frequency range from 8 to 12 Hz (4) for each separate channel.

$$D_c = \sum_{f=1}^4 P_c(f) \quad (3)$$

$$A_c = \sum_{f=8}^{12} P_c(f) \quad (4)$$

Subsequently one computes the delta alpha ratio of each separate channel as

$$\text{DAR}_c = D_c/A_c \quad (5)$$

after which the DAR_c of all channels n were averaged resulting in a global DAR.

$$\text{DAR} = \frac{1}{n} \sum_{c=1}^n \text{DAR}_c \quad (6)$$

To investigate whether it is possible to discriminate chronic stroke patients from healthy individuals based on their DAR and to investigate the influence of age, the DAR was calculated for each individual. Subsequently, since the sample size of the groups were unequal a non-parametric one-way between subjects ANOVA (Kruskal-Wallis Test) was applied, to compare the DAR between the different groups. If a difference was found, this test was followed by a post hoc analysis with Bonferroni corrections.

2.4.5 Spectral power deviations in chronic stroke patients

Power spectra were compared between chronic stroke patients and young and older healthy individuals using a wfANOVA. This provides information concerning the differences of relative contribution of different frequency bands between the groups. The power spectra were calculated as described before. However, the normalized power spectra of all channels were averaged in order to obtain one power spectrum per individual. These power spectra served as the input of a one-way between subjects wfANOVA (Appendix A).

To evaluate the complementary capacity of wfANOVA to the more common analysis of brain damage expressed by the DAR, the outcomes of the two analyses were compared. In addition, based on the results of the previous described analyses it was tried to find a method to refine the DAR.

Consistency and reliability of the DAR and power spectra

To determine whether the DAR and a one-way between subjects wfANOVA are consistent during a measurement, the analyses were done on each of the trials separately for each of the subjects. Subsequently, the outcomes of the consecutive trials were compared. For the DAR this was done using a Friedman Test for each group separately. If this test reveals significant difference, Wilcoxon Signed Rank Tests with Bonferroni correction were performed as Post-Hoc tests. To evaluate the consistency of a power spectrum within a measurement, per group the power spectra of the consecutive trials were compared using a repeated-measures wfANOVA.

To test the reliability of the DAR and power spectra, the outcomes of the test and retest of 6 healthy subjects were compared. For the DAR this was tested using a Wilcoxon signed rank test. The reliability of the power spectra was evaluated using a repeated-measures wfANOVA.

3 Results

Five minutes of artifact free resting-state EEG was analyzed for 8 young healthy individuals, 4 older healthy individuals and 4 chronic stroke patients. Clinical information of the participants can be found in Table 1.

Table 1 Clinical information of the participating chronic stroke patients.

Subject	Age(SD)	Affected hemisphere (L/R)	ARAT-score (0-57)	FMA-score (0-66)	Time post stroke
Young	24.0 (±2.4)	-	-	-	-
Elderly	57.8(±12.7)	-	-	-	-
Patients	67.5 (±6.5)	-	-	-	-
P1	64	R	5	13	7 years
P2	63	L	54	39	4 years
P3	77	R	51	62	8 months
P4	66	L	3	9	18 years

P = patient, L = left hemisphere, R = right hemisphere, ARAT = Action Research Arm Test, FMA = Brunnstrom Fugl Meyer Assessment (upper limb subtest).

BSI

The BSI was calculated for each of the young healthy individuals, healthy elderly and chronic stroke patients (Table 2). A Kruskal-Wallis Test indicated a significant difference between the groups ($\chi^2(2) = 8.487, p=0.014$). A Bonferroni post hoc test showed that the asymmetry calculated as the BSI did not differ significantly between healthy young individuals (Mdn=0.11, IQR=0.03) and healthy elderly (Mdn=0.11, IQR=0.004). However, the BSI was significantly increased in chronic stroke patients (Mdn=0.21, IQR=0.02, $p<0.001$) compared to both young and old healthy individuals (Figure 3).

In addition, asymmetry was investigated using a method in which the power spectra of the hemispheres were compared using a repeated-measures wfANOVA in which the electrodes were paired. Figure 4A shows an example of the power spectra of a young subject which was representative for all healthy individuals. The power spectra of the left electrodes do not seem to differ from the power spectra of the right electrodes. This was supported by the wfANOVA. The wavelets that describe the power spectra of the left electrodes did differ from those describing the power spectra of the right electrodes.

However, these differences were small and only present at a few individual frequencies instead of a larger part of a frequency band. The contrast line in Figure 4B reflects the wavelets which were significantly different between the left and right hemisphere. Moreover, when the outcomes of all healthy subjects were compared there was no frequency at which the difference was present for each of the healthy individuals (*Appendix B, Figure B1*).

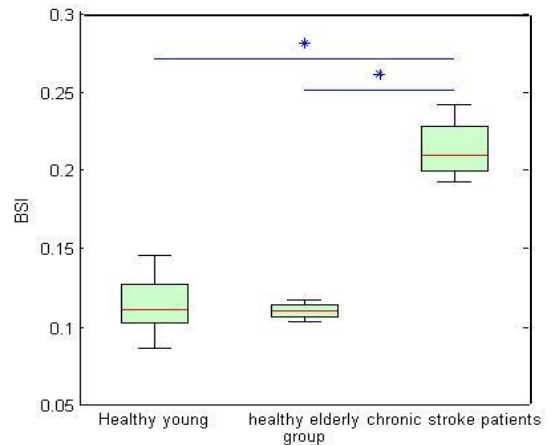


Figure 3 The Brain Symmetry Index of the three groups. A significant difference was found ($\chi^2(2) = 8.487, p = 0.014$). Post hoc tests with a Bonferroni correction showed that the BSI does not differ between young healthy individuals and healthy elderly. The BSI of chronic stroke patients is significantly higher compared to both control groups. * $p < 0.001$

Table 2 Global BSI, BSI_α and BSI_θ values of each group and of each individual stroke patient.

	N	BSI _{median} (IQR)	BSI _α (IQR)	BSI _θ (IQR)
Young	8	0.11 (0.03)	0.09 (0.03)	0.07 (0.02)
Elderly	4	0.11(0.004)	0.11 (0.01)	0.12 (0.04)
Patients	4	0.21 (0.02)	0.18 (0.04)	0.25 (0.07)
P1		0.20	0.15	0.32
P2		0.24	0.19	0.11
P3		0.19	0.24	0.24
P4		0.21	0.18	0.26

N = number of individuals per group, P = patient, BSI = global Brain Symmetry Index (1-25Hz), IQR = interquartile range. BSI_α = BSI of the alpha frequency band (8-12Hz), BSI_θ = BSI of the theta frequency band (4-8Hz)

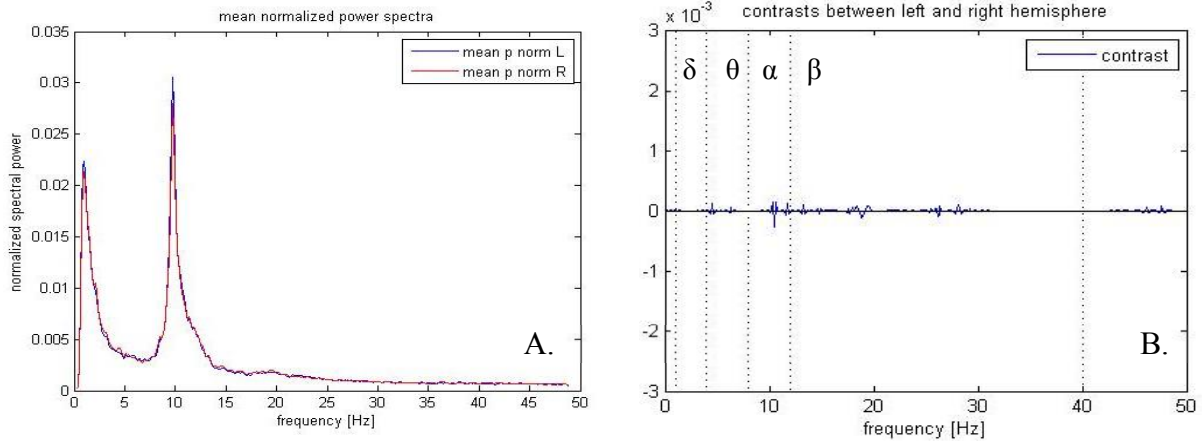


Figure 4 A. An example of the mean normalized power spectra of the electrodes above the left and right hemisphere of one of the healthy young individuals. These power spectra do not seem to differ between hemispheres. B. The corresponding contrast reflects the differences between the power spectra which were significant.

In contrast, in all four chronic stroke patients one or more differences were found between the power spectra of the left and right electrodes which covers at least one third of a frequency band. All patients showed a significant difference in their power spectra at the alpha frequency band (8-12Hz) (Figure 5). Moreover, in three out of four patients (P1, P2 and P4) the relative amount of alpha activity was decreased at the lesioned hemisphere. For this reason, the BSI was recalculated based on just the alpha band (Table 2). A Kruskal-Wallis Test indicated a significant difference in BSI_{α} between the groups ($\chi^2(2) = 7.428, p=0.024$). A post hoc test showed that the BSI_{α} of patients was significantly larger than young healthy individuals ($p<0.01$) and healthy elderly ($p<0.05$). Besides, all patients showed an asymmetry in their power spectra at the theta frequency band (4-8Hz). Therefore, a BSI based on the theta band was calculated (Table 2). A Kruskal-Wallis Test indicated a significant difference between the groups ($\chi^2(2) = 9.448, p=0.009$). A post hoc test showed that the BSI_{θ} of patients was significantly larger than the BSI_{θ} of young healthy individuals ($p<0.001$) and healthy elderly ($p<0.01$).

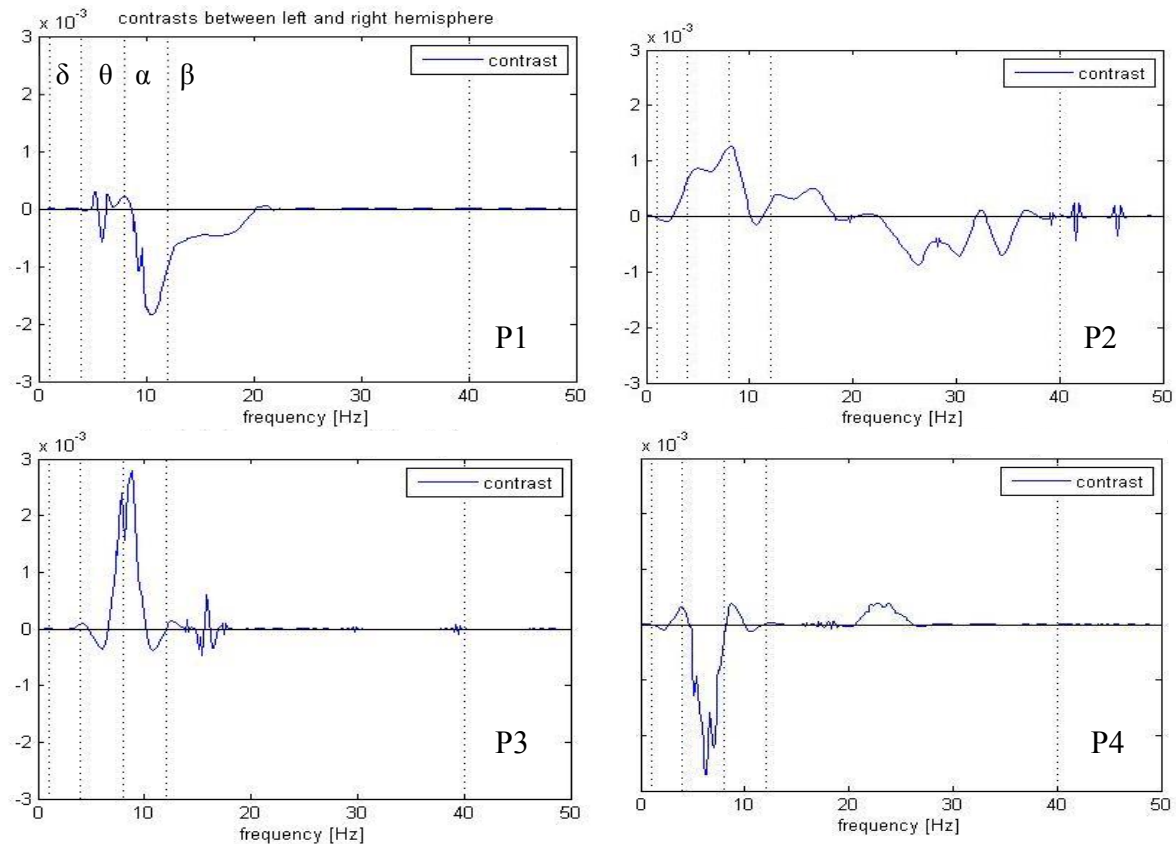


Figure 5 Comparison of the power spectra of the electrodes above the left and right hemisphere of four chronic stroke patients (P1-4) using a wfANOVA. Contrast reflects the differences between the power spectra which were significant. Positive values reflect higher relative power at the right hemisphere. Vertical lines separate the frequency bands.

Consistency of the BSI and the wfANOVA method within a measurement was tested by comparing the five trials. Friedman Tests indicated that the BSI did not differ between the trials within a measurement in each of the groups (young: $\chi^2(4)=4.667$, $p=0.32$; elderly: $\chi^2(4)=4.600$, $p=0.33$; patients: $\chi^2(4)=6.600$, $p=0.16$)(Figure B2). Also the asymmetry based on wfANOVA did not differ between the trials.

The reliability between measurements was investigated by a test-retest design using the Wilcoxon signed rank test. The BSI did not differ between the test and retest ($Z=-.105$, $p=0.917$)(Figure B3). Also the asymmetry analysed using a wfANOVA did not differ between the measurements.

DAR

The results concerning the DAR are shown in Table 3. A Kruskal-Wallis Test revealed that there were no statistically significant differences between groups, $\chi^2(2)=3.429$, $p=0.180$ (Figure 6).

Next, the power spectra of the young group were compared to those of the older individuals using a one-way between subjects wfANOVA. Although the power spectra do seem to differ between the groups (Figure 7), the wfANOVA showed that the power spectra of the chronic stroke patients are not significantly different from the power spectra of healthy individuals ($P>0.05$).

As the asymmetry analysis revealed that in all stroke patients of the current study the power spectra differed between the hemispheres especially at the alpha frequency band (Figure 6), the DAR will differ between the hemispheres. Therefore, the DAR was calculated for the lesioned and non-lesioned hemisphere separately (Table 3). Results show that in three out of four chronic stroke patients the DAR of the lesioned hemisphere is higher compared to their non-lesioned hemisphere. P3 showed an increased relative alpha power in the lesioned hemisphere, which corresponds to a lower DAR at the lesioned side. A Kruskal-Wallis Test was applied to compare the DAR_{lesioned} of the chronic stroke patients with the DAR of the healthy individuals. This test showed that there was no statistically significant difference between the groups, $\chi^2(2)=3.877$, $p=0.144$.

Table 3 DAR values of each group and of each individual stroke patient distinguishing lesioned and non-lesioned hemispheres.

	N	DAR _{median} (IQR)	DAR _{lesioned} (IQR)	DAR _{non-lesioned} (IQR)
Young	8	0.80 (0.46)	-	-
Elderly	4	1.29 (0.64)	-	-
Patients	4	1.65 (0.38)	1.53 (0.73)	1.30 (0.42)
P1		1.72	2.15	1.34
P2		1.57	1.78	1.26
P3		1.76	1.28	2.01
P4		0.66	0.74	0.58

N = number of individuals per group, P = patient, DAR = Delta Alpha Ratio, IQR = interquartile range.

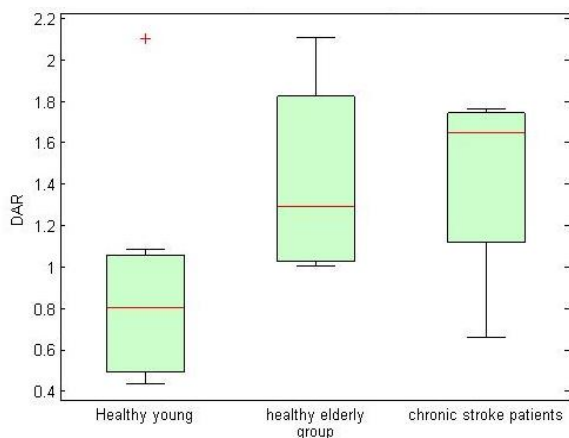


Figure 6 Delta Alpha Ratio of the healthy young individuals, healthy elderly and chronic stroke patients. $\chi^2(2) = 3.429$, $p = 0.180$.

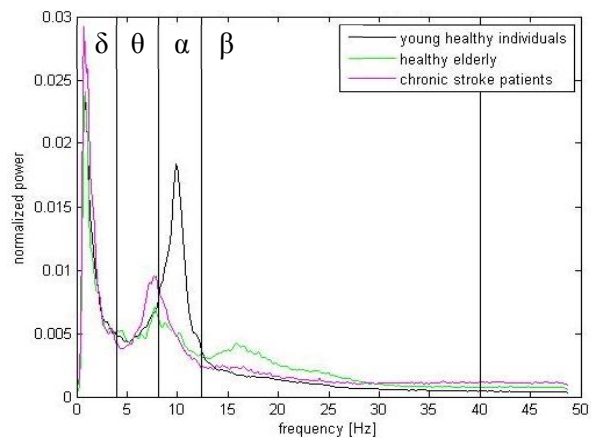


Figure 7 Normalized power spectra of the different groups. The wfANOVA showed that there were no significant differences.

Consistency of the DAR and the wfANOVA method within a measurement was tested by comparing the five trials of each subject. Friedman Tests indicated that the DAR did not differ between the trials within a measurement in the group of healthy young individuals and chronic stroke patients (young: $\chi^2(4)=3.597$, $p = 0.46$; patients: $\chi^2(4)=3.400$, $p=0.49$)(Figure B4), while a significant difference was found in the older healthy group ($\chi^2(4)=4.600$, $p=0.33$). However, Wilcoxon Signed-Rank Tests indicated that the DAR did not seem to differ between the consecutive trials or between the first and last trial. A repeated-measures wfANOVA revealed that the power spectra are not different between the trials.

The reliability between measurements was investigated using a test-retest design. A Wilcoxon Signed-Ranks Test showed that the DAR is not different between the test and retest ($Z=-.734$, $p=0.463$) (Figure B5). Besides, repeated-measures wfANOVA indicated that the power spectra did not differ significantly between the test and retest measurements.

4 Discussion

The prediction of functional recovery of the upper extremity after stroke and plasticity of the brain are of major interest for stroke survivors and caregivers. The current study was study of the 4D-EEG project, which consists of a cross-sectional and a longitudinal study. The main goal of the 4D-EEG project is to find parameters that improve prediction of functional recovery of the upper extremity. Prior to the longitudinal study of the 4D-EEG project, the cross-sectional data will be evaluated in order to find valid and reliable EEG parameters which may have a predictive value concerning functional recovery post stroke.

The analyses performed in the current study are based on part of the data set of the cross-sectional 4D-EEG study, as the measurements are not yet finished. Therefore, current results may be interpreted as a preliminary study. Nevertheless, it gave insight in the differences between chronic stroke patients and healthy individuals concerning the BSI and DAR, as well as the complementary capacity of the wfANOVA to these two parameters.

The first aim of the current study was to investigate whether the BSI and DAR differed between healthy individuals and chronic stroke patients.

BSI

As hypothesized, it is possible to discriminate chronic stroke patients from healthy individuals based on their BSI. To the best of the authors' knowledge this was not studied before. Since no differences were found between the BSI of young and older healthy individuals, it can be concluded that, at least in these groups of subjects, age does not influence asymmetry reflected by the BSI.

The BSI of healthy individuals has been investigated before by Van Putten and Tavy (2004), who reported an average value of $0.042(\pm 0.005)$. This value is lower compared to the BSI of healthy individuals calculated in the current study ($0.11(\pm 0.02)$). This discrepancy may possibly be caused by the different methods of data acquisition. Van Putten and Tavy measured EEG using a bipolar 8 channel subset (F4-C4, F3-C3, C4-P4, C3-P3, P4-O2, P3-O1, F4-T4 and F3-T3) resulting in only four channel pairs between the hemispheres, while in the current study a ground electrode was used and the BSI was calculated over 26 electrode pairs. Nevertheless, the present study showed a significant difference between healthy individuals and chronic stroke patients. This asymmetry may possibly be caused by for example irreversibly damaged brain tissue or increased activity of the contralateral hemisphere. Van Putten and Tavy (2004) investigated the BSI of stroke patients in the acute phase post stroke in comparison to healthy individuals. They concluded that it is possible to discriminate stroke patients from healthy individuals based on their BSI (Van Putten and Tavy, 2004). However, the current study is the first which shows that this is still possible in the chronic phase post stroke.

A range of BSI norm values at which no pathology may be expected is still lacking in the literature. A healthy BSI range may be estimated based on the mean BSI of the healthy individuals participating in this study plus two times the standard deviation. This results in a range from 0 (full symmetry) to 0.15 at which asymmetry is present while no pathology is expected. This boundary was supported by the data of the current study, as none of the healthy individuals and all chronic stroke patients showed a BSI above this value.

DAR

In contrast to the hypothesis, in the present group of subjects it was not possible to discriminate chronic stroke patients from healthy individuals based on the calculated DAR. The DAR of young healthy individuals did not significantly differ from the DAR of healthy elderly. Therefore, it was concluded that age most likely does not influence the DAR.

The DAR of healthy individuals as calculated in this study appears to be slightly lower compared to findings of Finnigan et al. (2007) who calculated a mean DAR of $1.58(\pm 0.93)$ based on EEG data of six healthy elderly, while we found a median value of $1.29(\pm 0.64)$. Based on their sample size, mean value and standard deviation, the confidence interval was calculated to be $CI_{95} = [0.84; 2.32]$. The DAR in the present study falls within this range, which indicates that it does not deviate from the finding of Finnigan et al. (2007). Nonetheless, there is a large discrepancy between the calculated DAR of stroke patients in the current study ($1.65(\pm 0.38)$) and the value calculated by Finnigan et al. (2007), who calculated a DAR of $5.63(\pm 3.74)$. This discrepancy may be explained by the fact that the stroke patients measured in the current study were in the chronic phase (≥ 6 months post stroke), while the stroke patients who participated in the study of Finnigan et al. (2007) were still in the sub-acute phase (48-52h post stroke). Therefore, it is not possible to compare the DAR values of these two studies.

Furthermore, Finnigan and Van Putten (2013) stated that it may be appropriate to consider a value of one or less to be relatively normal, while values higher than two may be considered abnormal. However, the CI_{95} of healthy individuals calculated based on the data of Finnigan et al. (2007) goes beyond this border. None of the healthy elderly who participated in the current study showed a DAR below one, while one individual even transcended the border of abnormality. In addition, one of the chronic stroke patients showed a DAR below one, while none of the patients exceeded a value of two. This also emphasizes the amount of natural variation among chronic patients. We therefore propose to redefine the borders in future research, which will discriminate healthy individuals and chronic stroke patients.

The fact that the current study could not discriminate chronic stroke patients from healthy individuals may also be due to the low number of individuals. Figure 6 shows a trend towards significant differences between these three groups. An increased sample size may have resulted in a significant difference. The number of individuals required to attain a significant difference was calculated as $n = (zs/e)^2$. In which $z = 1.96$ for a 95% confidence interval, $s = 0.5$ as the standard deviation and the desired margin of error e was set on 0.20, resulting in a sample size of 25. If all data of the cross-sectional 4D-EEG study is available, the data should be reanalysed in order to be draw conclusions on this parameter.

wfANOVA

The second aim of this study was to investigate whether a method in which the wavelet based functional ANOVA was used could be complementary to the BSI and DAR.

In order to analyze the asymmetry, the normalized power spectra of the left and right hemisphere were compared using a repeated-measures wfANOVA. The asymmetry analysis showed that the normalized power spectra of the healthy individuals was not different between the hemispheres, as the differences were small and present at only small parts of the frequency bands (Figure B6), except for one individual. Since the outcomes of young and older healthy individuals did not differ, as was hypothesized, it can be concluded that age does not influence asymmetry. In contrast, the normalized power spectra of the chronic stroke patients did differ between the hemispheres. The finding that in all chronic stroke patients an asymmetry was present at the alpha and theta band is in accordance with the hypothesis. However, asymmetry was also hypothesized to be present in the delta frequency band, which was not found in the present study.

The asymmetry at the alpha and theta band showed to be a consistent finding across the chronic stroke patients. All four chronic stroke patients showed a significant difference between the hemispheres at least at one-third of these frequency bands. In three out of four patients (P1, P2 and P4) the relative alpha activity was decreased at the lesioned hemisphere compared to the non-lesioned hemisphere. In three patients (P1, P3 and P4) the relative theta activity was increased at the lesioned hemisphere. These findings are in accordance to the results of Giaquinto et al. (1994), who showed that in stroke patients relative alpha and theta power were respectively lower and higher at the lesioned hemisphere compared to the non-lesioned hemisphere, in both acute and chronic phase.

The BSI and the analysis based on wfANOVA, showed an asymmetry in chronic stroke patients while there was no asymmetry observed in healthy individuals. Therefore they are concluded to be congruent. Moreover, the wfANOVA provides insight into the difference in relative spectral power between the hemispheres per frequency. Thereby, it provided insight into opportunities to augment discrimination between chronic stroke patients and healthy individuals concerning the BSI.

Based on the finding that in stroke patients the relative spectral power differed between the hemispheres at the alpha and theta frequency band, the BSI was recalculated based on these separate frequency bands. As can be seen in Figure B6, the BSI_{α} does not seem to improve the discrimination between healthy individuals and chronic stroke patients, while a BSI_{θ} is more likely to augment this discrimination. For both the chronic stroke patients and healthy individuals the BSI_{α} was slightly lower compared to the regular BSI (Table 2), and indeed did not improve the

discrimination. This finding is in contrast to the results of Juhasz et al. (1997), who showed lower absolute alpha power in the lesioned hemisphere in the acute phase post stroke in 52% of the patients. However, as the patients in the current study are chronic patients, this discrepancy may be due to changes during the recovery phase. In contrast, in chronic stroke patients the BSI_{θ} is higher compared to the regular BSI, while in healthy individuals the BSI_{θ} is lower compared to the BSI (Table 2). As it was shown that the BSI_{θ} increased the possibility of discrimination between healthy individuals and chronic stroke patients, the BSI_{θ} is an important refinement of the regular BSI. Therefore, the author suggests that an analysis based on the wfANOVA is complementary to the BSI.

The discrepancy between the BSI_{α} and the asymmetry at the alpha frequency band as shown by the wfANOVA, suggests a difference between the two ways of calculating asymmetry. This may be due to using normalized power spectra as input of the wfANOVA, while in the calculation of the BSI the absolute spectral power was used, which was not obtained from normalized power spectra. The reason for normalization is to overcome the influence of anatomical characteristics. Using the wfANOVA, standardization is necessary in order to be able to compare power spectra. However, if the overall brain activity of one of the hemispheres is higher, it may lead to an upward shift of power spectra, which will result in an increased BSI value. This difference in amount of brain activity will not be detected using the wfANOVA in which power spectra were normalized. Therefore, the different calculations may result in different outcomes concerning the asymmetry of the same individual. Though, the wfANOVA method is still meaningful as it provides insight into the differences between the hemispheres concerning the relative amount of activity at different frequency bands. Even though this study included only four chronic stroke patients, it can be concluded that the wfANOVA is complementary to the BSI. When the EEG of a larger number of chronic stroke patients is measured, these analyses should be repeated to be able to confirm the preliminary outcomes.

In addition, the wfANOVA was used to compare power spectra of chronic stroke patients and healthy individuals. The wfANOVA showed that, at least in these groups of subjects, the power spectra of chronic stroke patients did not differ from young and older healthy individuals. Furthermore, age did not seem to influence the power spectra.

It should be noted that besides the DAR also the wfANOVA method shows a trend towards a group difference (Figure 8). Compared to healthy elderly, chronic stroke patients seem to have an increased relative spectral power in the delta and theta frequency band and a decreased relative spectral power in the alpha frequency band. To the best of the authors' knowledge differences of spectral power over a frequency range from 1 to 49 Hz between stroke patients and healthy individuals have not been investigated before. Besides, a trend concerning an age related difference was shown. The relative spectral power in the alpha band seems to be decreased in the older healthy individuals compared to the young group as hypothesized. This is in accordance with Bazanova (2012) who showed that alpha power increases until puberty after which it is constant until the age of forty when it starts to decrease. This would underline the importance of an age-matched control group in a cross-sectional study in which EEG data is compared between groups. Moreover, also the wavelets which describe the power spectra show the trend of differences between the groups (Figure 9).

The findings of the wfANOVA method are in accordance with the outcomes of the DAR analysis and show similar trends. wfANOVA is suggested to be complementary to the DAR as the analysis based on wfANOVA provides insight into the differences between the groups concerning the relative contribution of each frequency to the total power, instead of just the ratio between delta and alpha power.

The asymmetry analysis based on the wfANOVA showed that the power spectra of stroke patients differed between the hemispheres especially at the alpha and theta frequency band (Figure 6). This is in accordance to a previous study, which showed that the relative alpha power was higher at the non-lesioned hemisphere (Giaquinto et al., 1997). This could result in a higher DAR at the lesioned hemisphere compared to the non-lesioned hemisphere, which could augment the discrimination of stroke patients and healthy individuals. Therefore, the DAR was also calculated per hemisphere. The DAR_{lesioned} did not result in a significant difference between the groups. Nonetheless, the mean value of the DAR_{lesioned} is higher compared to the regular DAR. This resulted in a decreased p-value, which increases the chance of discrimination between healthy individuals and chronic stroke patients in larger populations. Therefore, a DAR based on just the lesioned hemisphere may be a refinement. However, this should be confirmed by investigating a larger patient population.

For each of the investigated parameters no differences were found between the outcomes of the consecutive trials, the first and last trial or between the test and retest measurement. Therefore, it can be concluded that all investigated methods are robust and consistent within and between measurements.

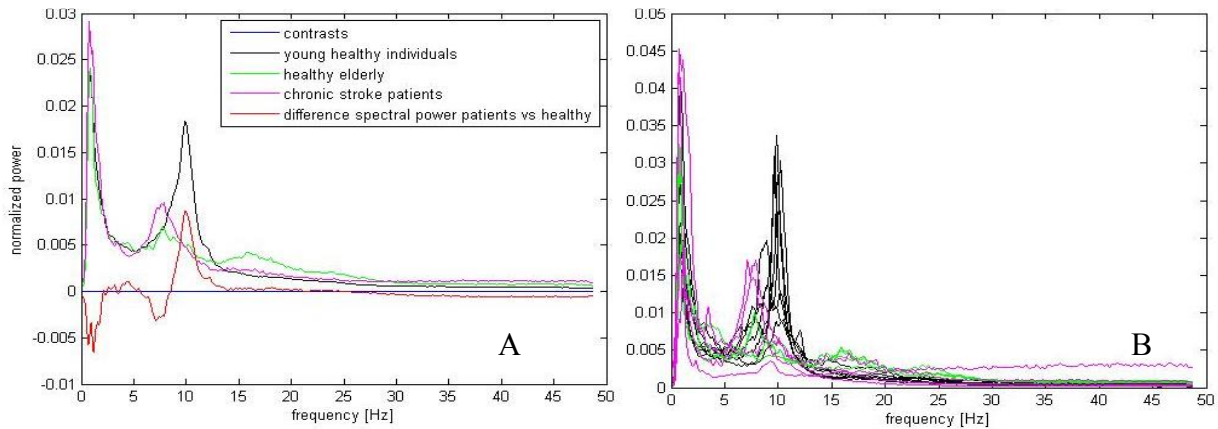


Figure 8 A. Power spectra of the different groups. ‘Contrasts’ showed that there is no significant difference of the wavelets between the groups. B. Mean power spectrum of each individual separately. Both figures show a trend toward significant differences between power spectra.

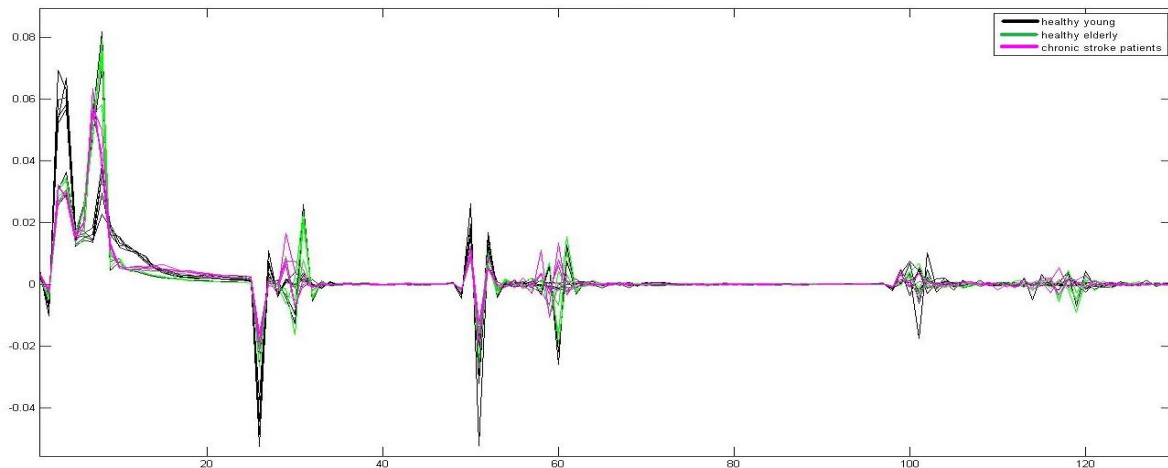


Figure 9 Wavelets of each group, in which the x-axis reflects the wavelet number while the y-axis is a reflection of the contribution of that wavelet to the waveform (power spectrum). No significant differences were found between the wavelets of different groups. However, this figure shows that there may be trends.

Limitations

The major limitation of the current study is the low number of subjects, especially in the chronic stroke patients group and the age-matched healthy control group. Therefore, the data will be reanalysed when all data of the cross-sectional study of the 4D-EEG project is available.

Besides, the resting-state data of the current study contained artefacts, which subsequently were removed during pre-processing. Artefacts as eye blinks and line noise were removed by respectively applying ICA and using band-stop filters. Although these filters are commonly used in EEG pre-processing, filters will affect the data and therefore may be considered as a limitation. However, artefacts caused by fascial muscle contractions or unexplained causes could only be removed from the data by removing a part of a trial. This resulted in less data than the total of five minutes as recorded. In the current study, it was attempt to avoid these artefacts by providing the participants clear instructions.

In addition, there are some limitations for the used calculation methods.

BSI

A limitation of the BSI is that some stroke patients may show similar impairments measured with the electrodes covering homologous regions of both hemispheres, while there was actually only damage in one of the hemispheres. This can be due to remote effects of a lesion. For example, higher power in the delta frequency range in all frontal electrodes. This may result in a ‘better’ BSI compared to patients having unilateral and hence less severe damage (Leon-Carrion et al. 2009).

DAR

A limitation of the DAR is that even in healthy individuals the DAR will not be equal at every part of the brain. This is caused by the fact that different brain regions show different dominant frequencies (Fingelkurts and Fingelkurts, 2010). If a channel was determined to be a 'bad channel' due to noise, it was removed from the analysis. However, depending on the amount and location of these channels, this may influence the outcome.

wfANOVA

To be able to compare power spectra between subjects, normalization is required. Structural differences as brain tissue and thickness of the skull between individuals and even between electrode locations may influence the measured power at the electrodes. In addition, although it was tried to keep the impedances low and equal, the impedances differed between electrodes, which may have influenced the measured local field potentials. For these reasons, the power spectra had to be normalized before they could be compared between individuals or between electrodes. However, not only structural variables influence the amplitude. A larger number of neurons oscillating at the same frequency, also leads to a higher amplitude measured at an electrode. Therefore, a difference in power could also indicate differences in neurophysiological activity, which is valuable information. However, a part of this information is lost due to normalization. As stated before, if the power is increased over the full range of the spectrum, this will lead to an increased BSI value, while comparing the power spectra after normalization will not show a difference at any frequency. However, if the power is increased at a specific frequency band, this may not be shown in the BSI. For this reason, the interpretation of asymmetry based on normalized power spectra may be different from the asymmetry calculated as the BSI.

Explanations concerning methodological considerations and choices made in the current study can be found in Appendix C.

Future research

The majority of studies in the field of recovery post stroke have been focusing on EEG in the (sub)acute phase (Finnigan and Van Putten, 2013). Therefore, the current literature lacks information concerning changes in EEG during the recovery period post stroke. In the longitudinal study of the 4D-EEG project, the EEG of stroke patients will be measured during the recovery period from the subacute phase to six months post stroke. Measurements will take place at week 1, 5, 12 and 26 post stroke. As brains are very plastic it will be of major importance to start measuring as early after stroke onset as possible. Neural plasticity, defined as the capability of the brain to alter function or structure in response to an event, is a crucial component of functional recovery after stroke (Pearson-Fuhrhop and Cramer, 2010). As neuro plasticity is reflected by changes, the availability of EEG data which includes both the acute and chronic phase is of major importance. Therefore, the current results could not provide information concerning neurophysiological changes. From this longitudinal data, the development of EEG-based parameters during the recovery period can be evaluated. This could provide insight into the neurophysiological recovery post stroke, as well as the relation between neurophysiological and functional recovery. Moreover, this data could be used to evaluate the capacity of the EEG-parameters to predict recovery of the upper extremity.

The asymmetry is suggested to be investigated in the longitudinal 4D-EEG study. A previous study showed a strong correlation between the BSI and clinical neurological condition quantified using the NIHSS in the acute phase post stroke (Van Putten and Tavy, 2004). In addition, it was shown that if a patient showed poor recovery the BSI increased (i.e. asymmetry increased) between one and three months post stroke. If a patient showed good recovery a decreased BSI was measured (Stojavic and Djurasic, 2013).

A study concerning the relative spectral power did also show changes over time. The relative delta and theta power respectively decreases and increases at the lesioned hemisphere during recovery, while it remained more or less constant at the non-lesioned hemisphere (Giaquinto et al., 1994). This may influence the asymmetry as measured using the wfANOVA.

Based on these previous studies, it is hypothesized that functional recovery of the upper limb will be accompanied by a decrease of asymmetry in brain activity reflected by a decreased BSI. Moreover, it is interesting to investigate at which frequency band the asymmetry changes during recovery using the wfANOVA. In addition, it is suggested to investigate whether the BSI may be used as a prediction parameter of functional recovery in longitudinal rehabilitation studies.

The DAR may also be an interesting parameter to investigate longitudinally. An animal study showed that the Alpha-to-Delta ratio (ADR, inverse DAR) was reduced in the acute phase in the penumbra of rats with experimentally induced stroke and gradually recovered to almost the same

ADR as before stroke onset in the chronic phase. This change in ADR correlated with recovery of motor function (Zhang et al., 2013). In addition, also the discrepancy in DAR values of chronic stroke patients of the present study and the subacute patients in the study of Finnigan et al. (2007) indicate the possibility that the DAR changes towards a normal value during recovery.

As the delta power at the lesioned hemisphere decreased during the recovery period, while the alpha power increased (Giaquinto et al., 1994), the DAR_{lesioned} is hypothesized to decrease over time. Giaquinto et al. (1994) did also show an increase of the relative theta power at the lesioned hemisphere.

However, in humans the development of the DAR over time from subacute to the chronic phase has not been studied before. The author suggests to investigate the spectral power at more frequency bands using the wfANOVA in a larger population. This will provide the possibility to investigate whether there are other ratios that could reflect neural deficits. To the best of the authors' knowledge, the study of Giaquinto et al. (1994) is the only study which investigated spectral power over time post stroke. The 4D-EEG project may confirm those results and will make it possible to correlate these changes with recovery status. A comparison between data of stroke patients and healthy individuals is still lacking. Furthermore, it is suggested to investigate whether the DAR and relative spectral power per frequency band may be used as a prediction parameter. This may all be investigated in the longitudinal study of the 4D-EEG project. Besides, the author proposes to distinguish between the lesioned and non-lesioned hemisphere in these analyses.

In the current study, in which only four chronic stroke patients were included, it was not possible to correlate the BSI or DAR with the upper limb capacity and motor function reflected by respectively the ARAT and FMA scores. However, the two EEG-parameters that augment the discrimination between chronic stroke patients and healthy individuals (BSI_{θ} and DAR_{lesioned}) do seem to show a trend towards a negative correlation (Figure B8, Figure B9). A higher BSI or DAR reflects more brain damage, resulting in lower scores at the clinical tests. However, for the DAR_{lesioned} this trend is only visible if P4 was considered to be an outlier (Figure B9b). The DAR_{lesioned} of this patient was 0.74, which is even lower than the lowest healthy individual. Based on the low number of patients, it was not possible to confirm the suggested association between the EEG-parameters and functional recovery. Therefore, it is suggested to reanalyse the data if all data of the cross-sectional study is available. However, these trends are promising for the longitudinal 4D-EEG study. It emphasizes the possible eligibility of these parameters as a predictor of functional recovery of the upper extremity.

Furthermore, a parameter that has not been investigated in the current study, but has been suggested to be changed due to stroke, is the alpha peak frequency (APF) (Giaquinto et al., 1994; Juhasz et al., 1997). The APF has been defined as the mean weighted frequency of the alpha band (Giaquinto et al., 1994). It has been shown that the APF is lower at the lesioned hemisphere compared to the non-lesioned hemisphere (Giaquinto et al., 1994). At the lesioned hemisphere it increases over time from the acute phase to 2-4 weeks post stroke (Juhasz et al., 1997). Whether this increase is parallel to clinical improvement is still unknown. Therefore, the author suggests to investigate the development of this parameter over time in relation to functional recovery. Furthermore it may be investigated whether the APF can contribute to prediction of functional recovery of the upper extremity post stroke.

Another promising line of research which unfortunately cannot be investigated in the 4D-EEG project, is the change in EEG within the acute phase post stroke. Finnigan et al. (2004) conducted an EEG measurement within sixteen hours after stroke onset followed by a second measurement within approximately eight hours after the first measurement. It was shown that a measure of the rate of change of average scalp delta power (acute delta change index) correlates with the 30-day NIHSS scores. Therefore, it would be of interest to investigate how EEG parameters change within the acute phase post stroke and whether this can predict functional recovery of the upper limb reflected by the FMA and ARAT.

5 Conclusions

It is possible to discriminate chronic stroke patients from healthy individuals based on the BSI, but not based on the DAR. However, trends toward significant differences were found for the DAR.

Conducting a wfANOVA method in order to analyze asymmetry by comparing the power spectra of the left and right hemisphere is complementary to the BSI as it provides insight into the difference in relative spectral power between the hemispheres. This study showed that in all four chronic stroke patients a difference can be found at the alpha and theta frequency band. A BSI based on just the theta frequency band improves discrimination of chronic stroke patients from healthy individuals, in contrast to a BSI based on just the alpha frequency band. In addition, this

study showed that separating the DAR calculation of the lesioned and non-lesioned hemisphere may improve discrimination between healthy individuals and chronic stroke patients.

Using a wfANOVA method to investigate the differences in power spectra between groups is complementary to the DAR as it provides insight into the differences between power spectra of individuals at a range wider than just the delta or alpha frequency band. Although these differences were not significant, trends were shown. The trends concerning the differences of the DAR and power spectra between groups should be investigated when more data is available.

Besides, it is concluded that age does not influence the BSI, DAR or the power spectra. However, trends were found for the DAR and relative spectral power. In addition, it is concluded that the calculations conducted in this study are robust and consistent within and between measurements.

A promising line of future research is the longitudinal analysis of EEG in stroke patients (4D-EEG project). This will give insight into the development of EEG-based parameters as the BSI, DAR and spectral power over time from the acute to the chronic phase post stroke. This information is still lacking in the literature. In addition, the relation between these parameters and functional recovery of the upper extremity should be investigated. This could provide insight concerning the relationship between neurophysiological changes and functional recovery. Moreover, it should be investigated whether these EEG-based parameters can improve prediction of functional recovery.

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Appendix A

Wavelet based functional ANOVA

This analysis method was developed by McKay et al. (2013). Their original files can be found at the institutional website of the authors: https://neurolab.gatech.edu/wp/wp-content/uploads/2012/08/wfANOVA_demo.zip. To use it as a repeated-measures wfANOVA with pairwise comparisons the MatLab script should look like:

```

data           = [p_norm_left; p_norm_right]; % or: [p_norm_test; p_norm_retest]
n_channels    = 26; % amount of channel pairs, or subjects in case of a repeated-measures test.
subject       = [(1:n_channels)';(1:n_channels)'];
condition     = [ones(n_channels,1); ones(n_channels,1)*2];
factors       = [condition subject];
performposthoc = [1 0];

[contrasts]    = wfANOVA (data, factors, performposthoc);
    
```

To use this function as a one-way between subjects wfANOVA the MatLab script should look like:

```

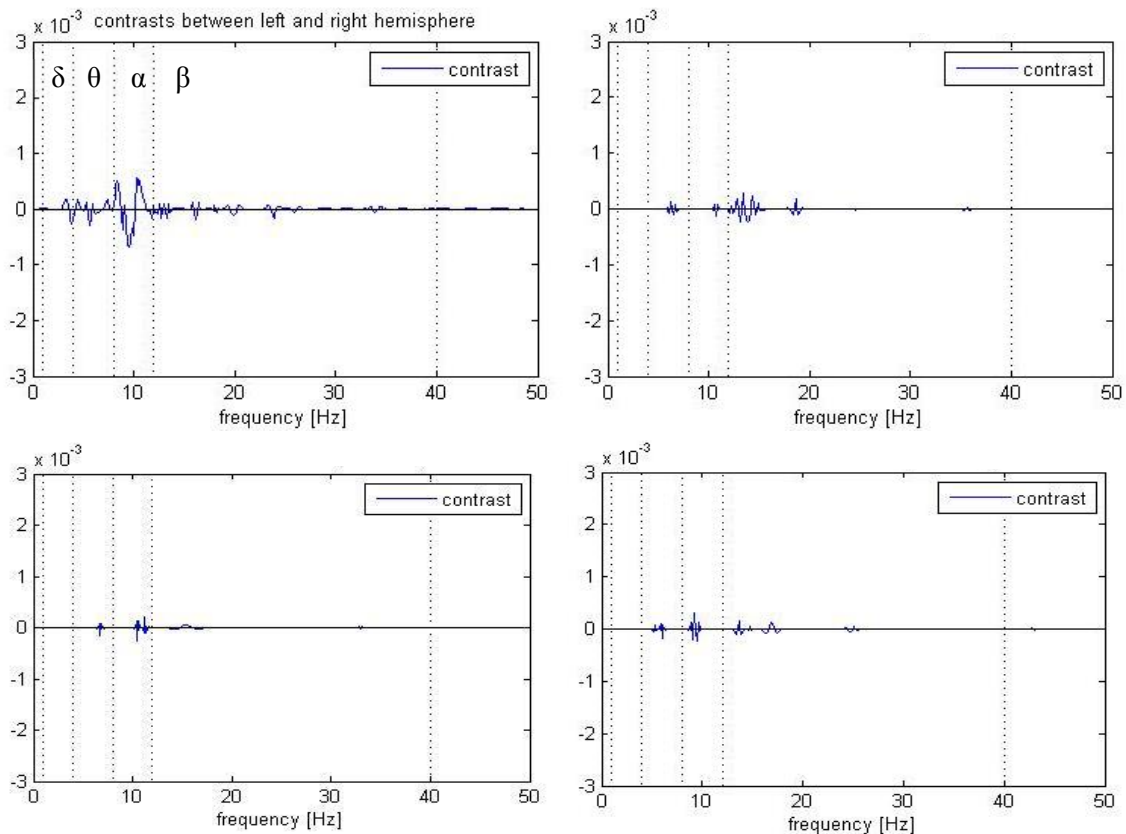
data           = [p_norm1; p_norm2 ];
nsub1          = 8; % amount of subjects in group 1
nsub2          = 4; % amount of subjects in group 2
subject        = [(1:(nsub1+nsub2))'];
condition      = [ones(nsub1,1); ones(nsub2,1)*2];
factors        = [condition subject];
performposthoc = [1 0];

[contrasts ]   = wfANOVA (data, factors, performposthoc);
    
```

Appendix B

Additional figures

Asymmetry was investigated using a wfANOVA method resulted in the following contrasts for the healthy individuals. Even for healthy individuals the power spectra of the hemispheres differed significantly. However, in most cases these differences were small and only present at a few individual frequencies, with one exception.



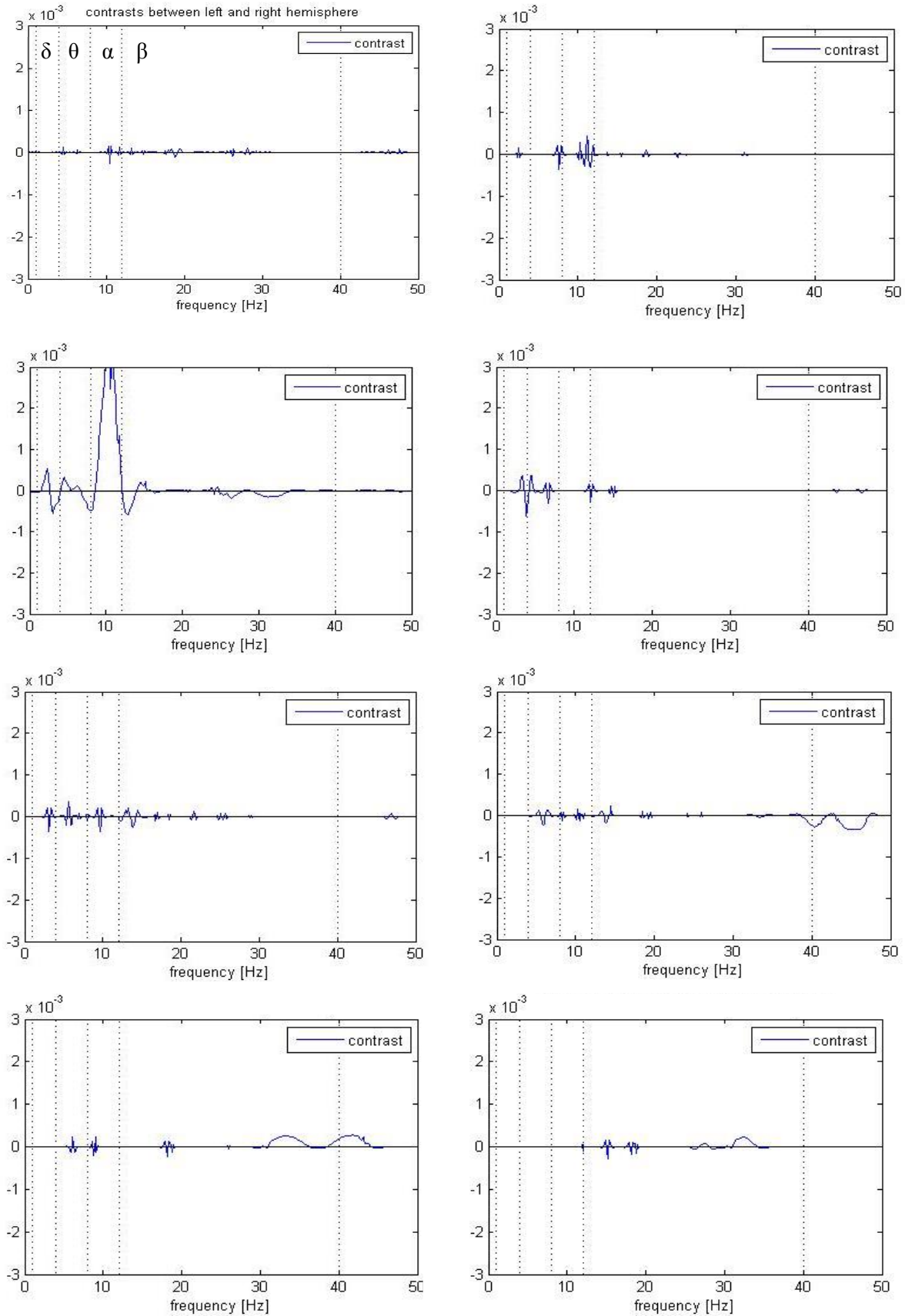


Figure B1 Asymmetry analysed using a wfANOVA method resulted in significant differences between the hemispheres. Most of the time these differences were small and present at a few individual frequencies instead of a large part of the frequency band, except for one individual. There was no frequency at which all individuals showed a significant difference.

Reliability of the BSI within and between measurements.

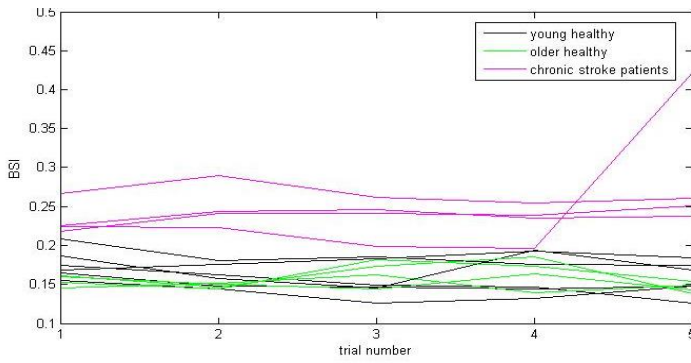


Figure B2 BSI within a measurement consisting of five trials. Each line represents one individual. Wilcoxon Signed-Ranks Tests indicated that the BSI did not differ between the consecutive trials within a measurement.

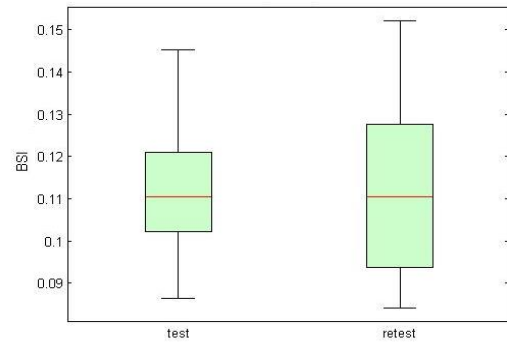


Figure B3 BSI test-retest. No significant difference was found between the test and retest of the BSI (Z=11, p=0.917).

Reliability of the DAR within and between measurements.

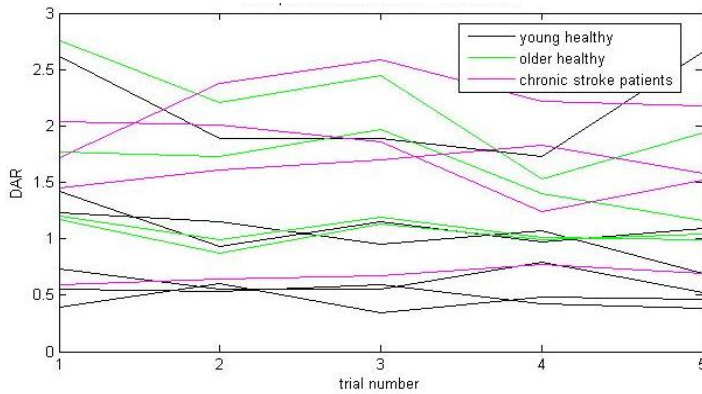


Figure B4 DAR of five trials within a measurement. Each line represents an individual. A Wilcoxon Signed-Ranks Test indicated that the DAR does not seem to differ between consecutive trials within a measurement.

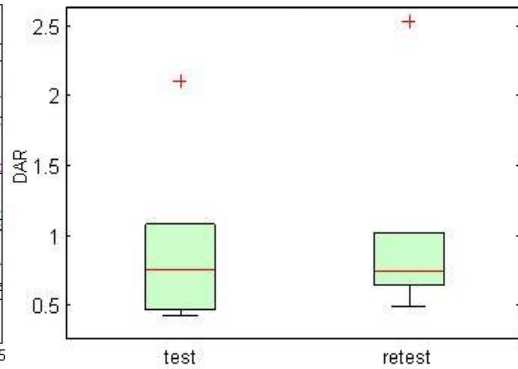


Figure B5 No significant difference was found between the test and retest of the DAR (Z=14, p=0.463).

BSI over a range from 1 to 25Hz.

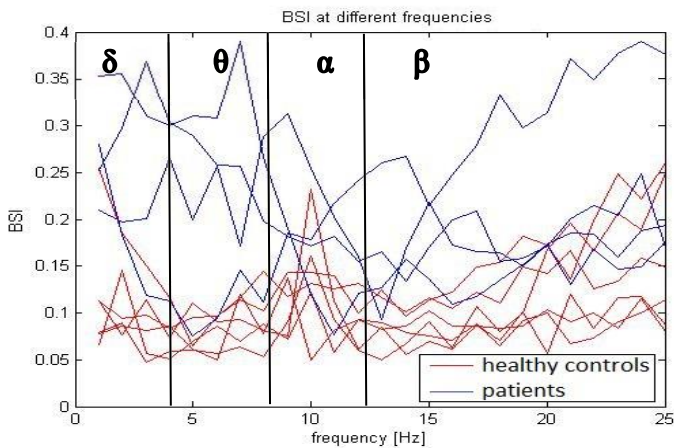


Figure B6 The BSI of healthy individuals and chronic stroke patients over a frequency range from 1 to 25 Hz. A BSI based on the alpha band only (8-12Hz) did not improve the discrimination between chronic stroke patients and healthy individuals, while a BSI based on the theta band only (4-8Hz) did.

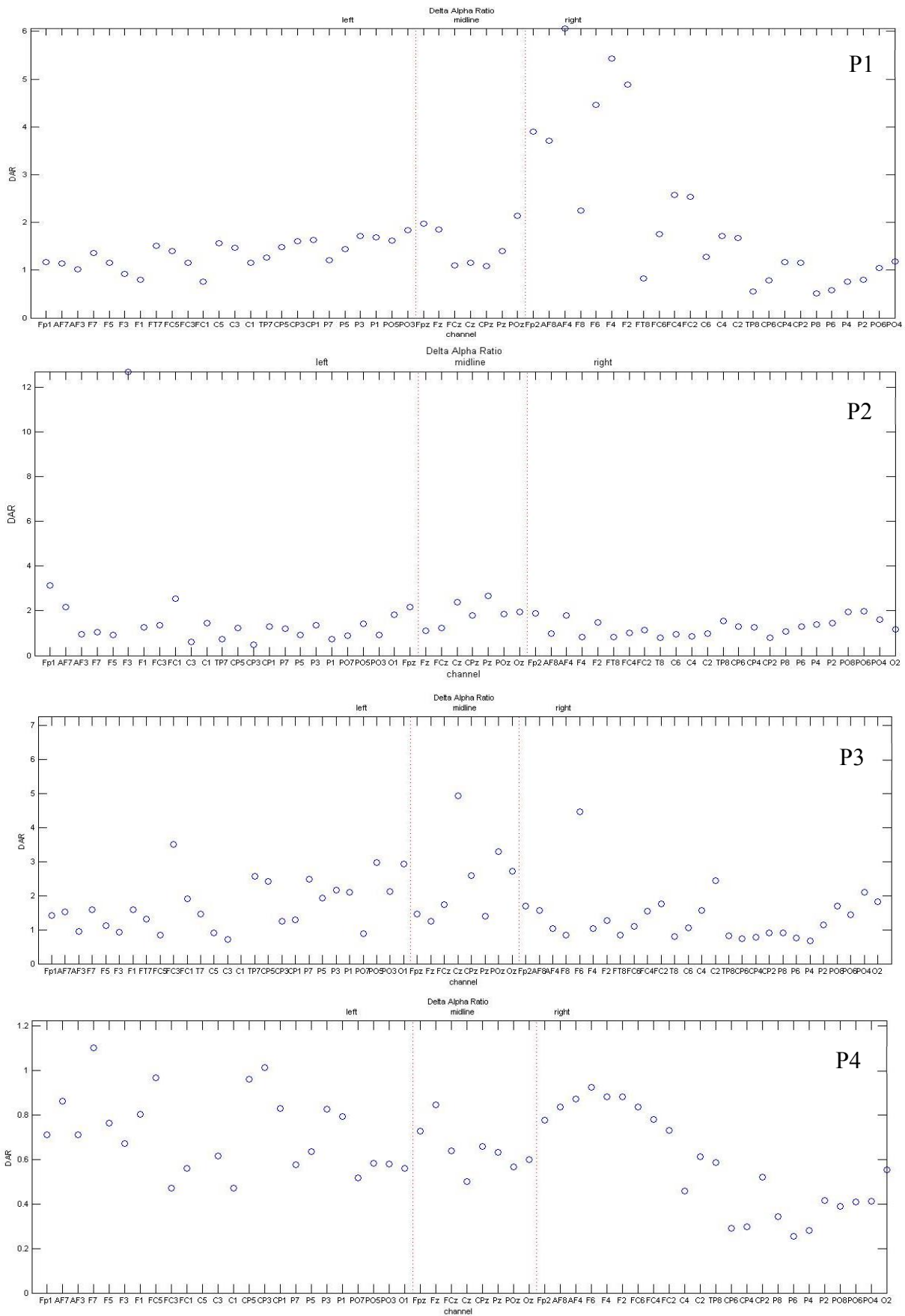


Figure B7 DAR per channel for each of the chronic stroke patients. Vertical lines separate the electrodes above the left hemisphere, the midline and the right hemisphere. In P1, P3 and P4 a clear difference can be seen in the DAR of the different hemispheres. P2 contains an outlier which could be the cause of the observed difference.

Trend lines between the EEG-parameters and clinical test scores.

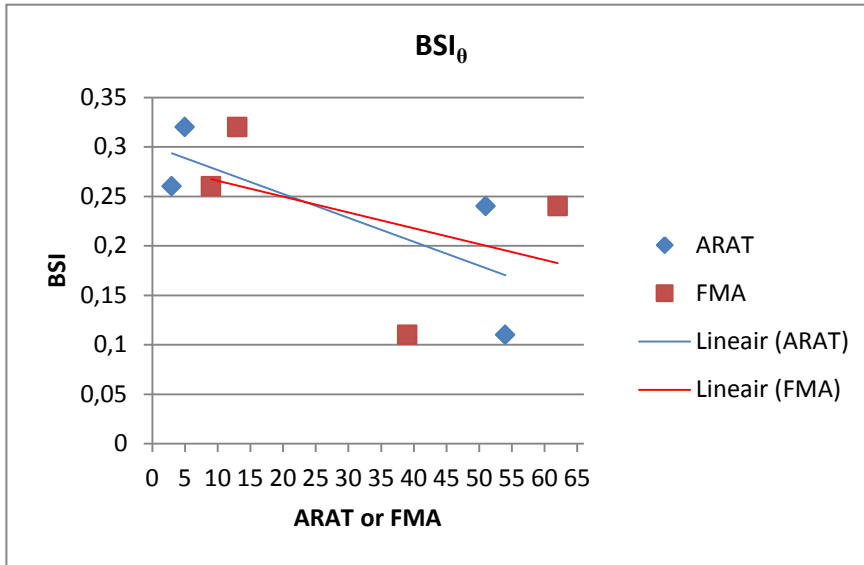


Figure B8 Trend lines between BSI₀ and ARAT, and between BSI₀ and FMA of the chronic stroke patients (N=4). Dots represent data points, lines represent linear trend lines.

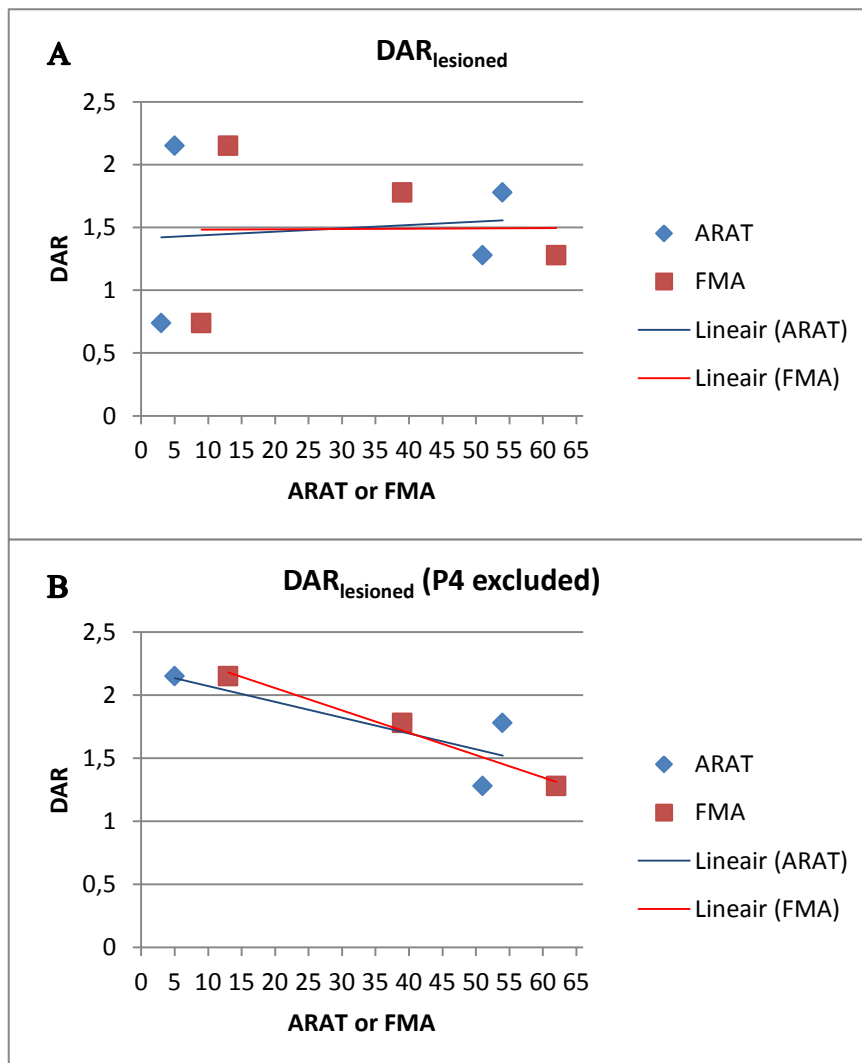


Figure B9 Trend lines between DAR_{lesioned} and ARAT, and between DAR_{lesioned} and FMA of the chronic stroke patients. Dots represent data points, lines represent linear trend lines. A. All chronic stroke patients (N=4). B. P4 (DAR_{lesioned} = 0.74) excluded (N=3), as it may be considered to be an outlier.

Appendix C

Methodological choices and recommendations

BSI

As mentioned before, increased power due to other causes than increased brain activity may bias the BSI. Focal seizures for example, referring to rhythmic muscle contractions of unilateral facial muscles, will increase the BSI (Van Putten and Tavy, 2004). This underlines the importance of accurate observations during the measurement.

In the calculation of the BSI, spectral power was used to calculate the BSI after which it was averaged over the frequencies and electrode pairs. However, averaging implies that there is a possibility of a normal distribution. This is not the case when spectral power was used because spectral power can range from zero to infinity. Therefore, normally the spectral power will be log-transformed before they will be used. By a log-transformation the spectral power will be distributed around zero, which will enable a normal distribution. For this reason it is proposed to investigate whether a log-transformed power spectrum should be used for the calculation of the BSI.

Something that should be considered carefully is the way of calculating the BSI. The BSI can be calculated as was done in the current study, by first calculating the BSI of each channel-pair, after which these values are averaged. This calculation was based on the calculation method using by Van Putten and Tavy (2004). Another way of calculating the BSI is by calculating the average spectral density over each hemisphere, after which the difference was calculated (Van Putten et al., 2004). If the power of the lesioned hemisphere is lower compared to the non-lesioned hemisphere at every channel-pair, there will be no difference in outcome. However, if the power is at some channel-pairs higher at one hemisphere and at other channel-pairs higher at the other hemisphere, these two calculations will result in different outcomes. It should be analysed whether this influences the results and discrimination between healthy individuals and stroke patients.

DAR

Also the DAR can be calculated in different ways. In the current study the DAR was calculated as the DAR of each electrode separately after which it was averaged over all electrodes as was done by Finnigan and van Putten (2013). Another way of calculating the DAR is by first averaging the power spectra of all electrodes after which the DAR was calculated based on one mean power spectrum (Finnigan et al., 2004; Finnigan et al., 2007). These two ways of calculating lead to different outcomes. One major disadvantage of calculating the DAR based on the average power spectrum is that electrodes which have a higher spectral power will predominate the DAR value. This can be overcome by normalization of the power spectra of the electrodes before averaging. Moreover, the advantage of the first calculation method is the possibility to look at the DAR per brain location. Therefore, in the current study this method was used to calculate the DAR.

The DAR was calculated per channel where after they were averaged. Averaging assumes uniform distribution. However, Figure B7 shows that this is not always the case. For example in the case of P2, which contains an outlier at the lesioned side. Therefore, it is proposed to consider the use of the median instead of the mean in the calculation of the DAR. However, it has not been investigated to which extent this influenced the results.

wfANOVA

Wavelet based functional ANOVA was used to be able to compare the normalized power spectra between the hemispheres or between individuals for each frequency while avoiding the multiple comparisons problem. A more simple method to compare power spectra is by using a two-sample Kolmogorov-Smirnov test. Using this test, it can be investigated whether the distribution of two power spectra are similar. However, if this test shows that the distribution of the power spectra is not equal, contrary to the wfANOVA it does not give information about the frequency band at which the discrepancy is located. This emphasizes the value of a wfANOVA.

Normally, the power spectra will be log-transformed before they will be used in statistical tests. This is because spectral power can range from zero to infinity, which makes a normal distribution impossible. By a log-transformation the spectral power will be distributed around zero. This will enable a normal distribution, which is important in statistics. However, in this study the power spectra were transformed to the wavelet domain where after the wavelets were compared. Log-transforming the power spectra will result in a function which is not as quickly converging as a normal power spectrum (Figure C1). Therefore, it will require more wavelets to describe a log-transformed power spectrum, consequently more tests should be done. For this reason the

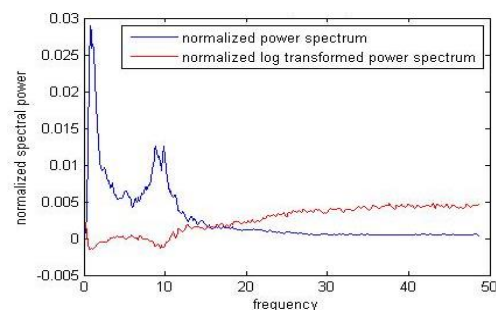


Figure C1 An example of a normalized power spectrum. A normalized power spectrum is more quickly converging compared to a log-transformed power spectrum. Therefore, the former can be described with less wavelets, resulting in a lower number of comparisons in the wfANOVA.

untransformed normalised power spectra were used as input of the wfANOVA.

The wfANOVA as developed by McKay et al. (2013) is based on 3rd order coiflet-type wavelets. However, there are several other possible wavelet types which can be eligible to use for the description of power spectra. Wei et al. (1997) showed that coiflets are excellent when approximating the sampling of a smooth function. As a power spectrum seems to be a smooth function, coiflet-type wavelets are assumed to be a good choice. However, whether other wavelet types or other order coiflet-type wavelets are better to describe power spectra should be investigated.

In order to be able to compare power spectra, they should be normalized. In the current study a normalization technique as dividing the power spectra by the total power was used. However, one should be careful using such a technique. It can for example result in 'mixing' in band specific brain results to all other bands. Therefore, the normalization of a power spectrum will be dependent on the curve of the power spectrum that was normalized, for example with a high power at a certain frequency band. Another option is to normalize by dividing the power by the total power in higher frequency bands, in which the power is more attributable to unspecific noise and less to brain activity. However, these frequency bands can also consist of power due to muscle activity when a subject was not completely relaxed. In this study the amount of muscle activity was not measured. Therefore, the first normalization technique was used. In future studies it is recommended to measure muscle activity of the neck muscles to be able to correct for this and to be able to use the second normalization technique to see whether this leads to different results.

In conclusion, the input of the ANOVA in this wfANOVA function are wavelets. In this function it has not been checked whether the wavelets are normally distributed and whether there is homogeneity of variances. When these assumptions are violated a non-parametric test should be used instead of the ANOVA. For the asymmetry calculation per individual a Friedman test should be implemented to be able to execute a dependent non-parametric repeated-measures ANOVA. For the analysis of the differences in power spectra between individuals the implementation of an independent non-parametric ANOVA as the Kruskal-Wallis will be proposed.

Appendix D

Additional analyses – Functional connectivity

In the rehabilitation medicine there is a demand for a way to relate arm function recovery to brain activity. Besides the evaluation of the brain symmetry index, delta alpha ratio and analyses based on wfANOVA, the author of this study has initiated the development of an analysis concerning functional connectivity in stroke patients. Therefore, this appendix will focus on functional connectivity, which can be calculated from resting state EEG data and may be an interesting variable to investigate in the longitudinal 4D-EEG study. However, it was not tested on all participants. Therefore, the author was not able to refine the method or draw any conclusions. Nonetheless, it deserves further study.

Structural damage caused by stroke is focal rather than diffuse. Though, due to physiological impairments in distributed functional networks stroke is associated with both local and global changes in brain function (Carter et al., 2012a). Recovery of function after stroke is largely due to reorganization of activity in existing (sub)cortical networks. Therefore, a connectivity based approach will provide insight into network reorganization during recovery (Carter et al., 2012a). The reorganization is not only visible when the networks are active, but also in resting-state networks. For this reason, resting-state activity may relate to behavioural deficits (Carter et al., 2012a).

Based on the relation between brain activity and arm function five regions of interest were chosen; the left premotor area (PMA_L), right premotor area (PMA_R), left primary motor cortex (M1_L), right primary motor cortex (M1_R) and the supplementary motor area (SMA). It was tried to create a method to quantify the connectivity between these five regions of interests. A way of quantifying this is by calculating the functional connectivity (FC) between these regions (Nolte 2004). FC is defined as the similarity in shape of the frequency spectra of the time series between spatially remote neurophysiological processes in rest (Carter et al., 2012a). One of the most simple ways of reflecting connectivity is by calculating coherence (Nolte et al., 2004), which is the modulus of coherency. However, because of the fact that electrodes on the scalp are close to each other a source of activity will be measured by several electrodes. This is caused by volume conduction. In this way the activity seems to be caused by multiple sources which are active at the same time in phase or anti-phase, instead of just one source of activity. However, when two brain regions are functionally connected, they will not be active at exactly the same moment. To be sure that the connectivity is caused by two different sources, there should be a phase lag. This information is not located in the modulus but in the imaginary part of coherency (Nolte et al., 2004). Therefore, the imaginary part of coherency was defined as the functional connectivity.

Corticospinal tract damage results in decreased interhemispheric resting-state FC (Carter et al., 2012b). In accordance with this, Zhang et al. (2014) stated that the resting-state FC between the ipsilesional M1 and the contralesional motor cortex was decreased in acute stroke, followed by a gradually increase during the recovery process. Eventually, in complete recovered patients the normal resting-state FC level was reached or even exceeded (Zhang et al., 2014). This positive association between resting-state FC and the amount of functional recovery is supported by Urbin et al. (2014) who found that the strength of homo- and heterotopic resting-state FC was lower in chronic stroke patients who still suffer from hemiparesis compared to healthy controls. In addition, patients showing stronger interhemispheric connectivity were less impaired (Urbin et al., 2014; Chen and Schlaug 2013). The interhemispheric connectivity is not the only process which influences the upper limb function. A general concept is that successful control of M1 by the ipsilateral motor control areas is important for upper limb function (Grefkes et al., 2008). Grefkes and colleagues concluded that a dysfunctional interaction between SMA and M1 may contribute to motor disability because of the loss of driving input exerted by the SMA (Grefkes et al., 2008).

Between the five regions of interest a network of ten connections can be formed. For each of these connections the FC can be calculated. The Functional Connectivity has not yet been investigated using this analysis design. In future research it should be investigated whether this way of analysing Functional Connectivity makes it possible to discriminate stroke patients from healthy individuals and whether it changes during the recovery period of stroke patients.

After the calculation on electrode level the calculation can also be conducted on source level. In this study the position of the electrodes and the anatomical landmarks of the nasion and both pre-auricular points were digitized using a 3D infrared camera system (Xensor 3D Electrode Digitizer) before EEG was recorded, to localize the electrodes and to be able to match the electrode position with the anatomical MRI. A MRI of the head of each participant was made in the VU Medical Centre using a 3 Tesla whole body MR scanner. This resulted in a high-resolution T1-weighted structural image. During the MRI measurement all subjects were instructed to remain as motionless as possible, foam padding was used to minimize head motion. Besides, earplugs were used to reduce scanner. In this way for each region of interest a source can be localized instead of the calculation based on the electrodes covering these regions of interest which is quite arbitrary.

Overall Functional Connectivity

Calculation of functional connectivity between all electrodes was done using the following Matlab code: First a frequency analysis using the FieldTrip Toolbox (Oostenveld et al., 2010).

```
cfg = [];
cfg.output = 'powandcsd';
cfg.method = 'mtmfft';
cfg.foolim = [1 100];
cfg.taper = 'hanning';
cfg.keeptrials = 'no';
freq = ft_freqanalysis(cfg, RS_clean);
```

In this code 'RS_clean' corresponds to artifact-free resting-state EEG data. After this frequency analysis functional connectivity can be calculated as the absolute value of the imaginary part of coherency.

```
for i = 1:length(freq.freq)
    for j=1:length(freq.labelcmb)
        Cab(j,i) = freq.crsspctrm(j,i);
        Pa(1,i) = freq.powspctrm(I.(cell2mat(freq.labelcmb(j,2))),i);
        Pb(j,i) = freq.powspctrm(I.(cell2mat(freq.labelcmb(j,1))),i);
        coherency_matrix(j,i) = Cab(j,i)/sqrt(Pa(i) * Pb(j,i));
    end
end
coherency_imaginarypart_absolute_mean = mean(abs(imag(coherency_matrix)));
```

Functional Connectivity per region of interest

Electrodes per region of interest (Figure D1):

- C1-C3-C5-FC3-CP3 = left primary motor cortex (M1_L), bright blue.
- C2-C4-C6-FC4-CP4 = right primary motor cortex (M1_R), dark blue.
- FC1-FC5-F1-F3-F5 = left premotor area (PMA_L), yellow.
- FC2-FC6-F2-F4-F6 = right premotor area (PMA_R), orange.
- FCz = supplementary motor area (SMA), green.

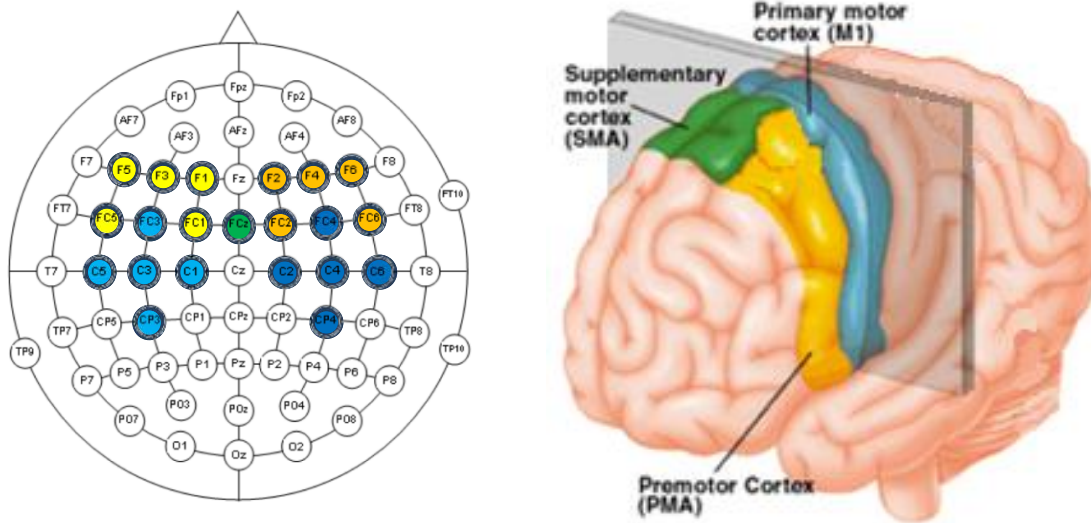


Figure D1 Electrodes covering the regions of interest based on a template brain. Yellow = left premotor cortex, clear blue = left primary motor cortex, green = supplementary motor cortex, orange = right premotor cortex, dark blue = right primary motor cortex.

Network

The network of five ROI's consists of ten connections (Figure D2). The FC of a connection can be found by averaging the FC of all possible connections between the two regions of interest of that connection. For example, the FC between the left and right primary motor area (M1) will be the average of the twenty-five possible connections between these two ROI's. The value of the FC will be compared to the overall functional connectivity between all electrodes. If a line turns red, this connections is not significantly different from this baseline value. If it turns green, it is significantly increased or decreased compared to the baseline value. The diameter of the dots reflect the mean power measured at the electrodes covering that ROI. A larger power will be reflected by a larger diameter.

Functional Connectivity

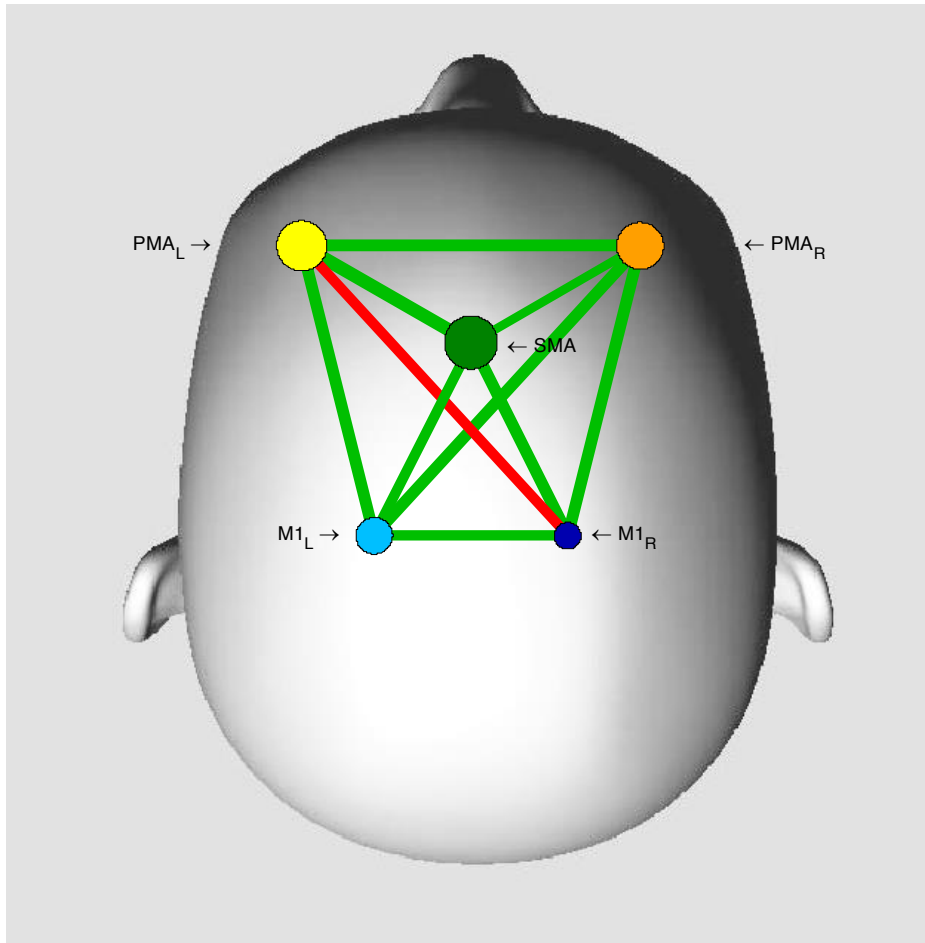


Figure D2 Network projected on a head, in order to display the network more clearly. The location of the dots which reflect the regions of interest do not correspond to the exact location of the regions of interest. Green lines correspond to functional connectivity between ROIs which is significantly different from overall functional connectivity. Red lines correspond to non-significant functional connectivity. Larger diameter of the circles reflect larger average power at that ROI.