# The influence of exercising on hippocampal volume in elderly with Mild Cognitive Impairment (MCI)

## A pilot study

**Ida van de Water**

**13-6-2018**

Bachelorscriptie Mens en Techniek|Bewegingstechnologie

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# Abstract

An increasing life expectancy brings higher risks for getting a neurodegenerative disorder like Alzheimer's disease (AD). As there is still no cure for this disease, expenses in health care keep rising because most of the care for patients with AD are from nursing homes and hospitals. The change from healthy aging to AD sometimes has an in between state, known as mild cognitive impairment (MCI). In that state, patients do have problems with their memory but are not limited in their activities of daily life. Slowing down the MCI state or preventing the onset of AD could be very useful to reduce the costs for this disease.

As the hippocampus is responsible for, among others, memory, the memory problems in MCI patients are probably due to a loss of hippocampal volume.
In healthy elderly, exercising seems as a way to prevent age related atrophy of the hippocampus and their related subfields (e.g. dentate gyrus). In MCI patients, the atrophy rate is even larger than in healthy elderly but not as large as in AD patients, contributing to the idea that MCI is a state between healthy aging and AD.
However, little literature exists about the influence of exercising on volumes of the hippocampus in a MCI population. Therefore, it is interesting to investigate what influence exercising could have on hippocampal volume in a MCI population, given the positive results in healthy elderly.

This study was a part of the NeuroExercise study, where participants with MCI exercise for 12 months according to a structured exercise program. At the beginning of the study and halfway (6 months into the program) a MRI scan was made to be able to look at the differences in brain volumes after half a year of exercising. The volumes of the hippocampus and dentate gyrus are determined, as in the dentate gyrus neurogenesis could possibly be stimulated by exercising.
17 participants had two MRI's (at 0 and 6 months) and their scans were analysed in the program FreeSurfer. FreeSurfer is software based on in- and ex-vivo data and shown to have accuracy and sufficient sensitivity in detecting changes in non-cortical structures.

With a multiple regression the relation between the dependent variables and the independent variables were determined. The dependent variables were the change in volume in hippocampus and dentate gyrus after 6 months in the program (Δ volume). The independent variables were the exercise groups (aerobic, anaerobic or control), the amount of exercise classes followed in 6 months and activity level measured with an Actiwatch.

The regression analysis does not show a statistical relation between the dependent and independent variables. However, only the dentate gyrus seems to benefit from the amount of exercise classes, but far from significant ($p = 0{,}191$ for the left dentate gyrus and $p = 0{,}157$ for the right dentate gyrus). The correlation between hippocampal volume at T0 and age is expected to be negative (i.e. higher age, lower hippocampal volume) because of the age related atrophy of the brain. This correlation was only minimal ($R^2 = 0{,}271$ for the left side and $R^2 = 0{,}040$ for the right side).
When looking at the influence of the amount of exercises on the change of volume, it is seen that the volumes of the right hippocampus and dentate gyrus remain at a stable level or increase when participants exercise less than a 100 times in 6 months.

These results thus suggest that the amount of exercising, when below the threshold of a 100 times in 6 months, keep the volumes of the right hippocampus and dentate gyrus at a stable level or increase the volumes. However, these findings are not statistically significant and further research is needed to confirm this relation.

# Content

# Introduction

According to the Alzheimer's Association, Alzheimer's disease (AD) is currently the most common neurodegenerative disorder. In the United States (US), every 66 seconds someone is diagnosed with AD, the most common form of dementia. It is even estimated that by 2050, every 33 seconds someone is diagnosed with AD (Association, 2017). Healthcare expenses are also increasing, as AD is one of the most expensive conditions. It is estimated that by 2018, the threshold of 1 trillion US dollars is reached (Wimo et al., 2017).

The decline in (cognitive) functioning from healthy aging to AD progresses over time. When there are deficits in cognitive functioning but the activities of daily life (ADL) are not impaired, elderly may be diagnosed with mild cognitive impairment (MCI) (Langa & Levine, 2014). There are 2 subtypes of MCI, amnestic MCI (aMCI), in which memory impairments are the main issue and non-amnestic MCI (naMCI), when other cognitive domains are impaired (Smith & Bondi, 2013, p. 76). These subtypes can be further divided in single and multiple domain impairments.
MCI is frequently diagnosed with the Mini Mental State Exam (MMSE), but the Montreal Cognitive Assessment (MoCA) shows greater sensitivity and specificity than the MMSE (Dong et al., 2012; Nasreddine et al., 2005). To diagnose MCI due to AD, the criteria according to Albert et al. (2011) is recommended for clinical trials (Albert et al., 2011). The study of Albert et al. (2011) holds guidelines on how to assess the probability of MCI due to AD and how likely the diagnosis of MCI can be made.

MCI may be a state between healthy aging and AD. However, not everyone with MCI progresses to AD. Some MCI patients go back to their normal level of cognitive functioning while others stay in the MCI state. Nevertheless, patients with aMCI are more likely to convert to AD than naMCI patients (Smith & Bondi, 2013, p. 74). Diagnostic criteria from the Diagnostic and Statistical Manual of Mental Disorders (D.S.M.) version V is used for the distinction between MCI and AD (American Psychiatric Association. & American Psychiatric Association. DSM-5 Task Force., 2013). The DSM V states that a patient can be diagnosed with AD when there are severe cognitive deficits in multiple domains and an impairment in the ADL.

To prevent MCI patients from progressing to AD, treatment options have shifted from pharmacological to non-pharmacological, as pharmacological treatments have been unsuccessful (Rodakowski, Saghafi, Butters, & Skidmore, 2015).
Currently known, the largest risk factor for non-familiar AD is age. However, age is not modifiable but another important risk factor for AD that is more modifiable, is physical inactivity (Laurin, Verreault, Lindsay, MacPherson, & Rockwood, 2001; Xu et al., 2015). Guidelines for the treatment of MCI recently published that exercising for at least twice a week has cognitive benefits for MCI patients (J. N. Barnes, 2015; Petersen et al., 2018). Exercising might thus be used as a non-pharmacological intervention.
Furthermore, exercising has more than only cognitive benefits. Especially in the elderly population, exercise has benefits on the general health as well (McPhee et al., 2016). Many studies that used exercise as an intervention used healthy elderly instead of MCI patients. Literature is limited and heterogenic on the effect of exercising in patients with MCI (Ahlskog, Geda, Graff-Radford, & Petersen, 2011; Bherer, Erickson, & Liu-Ambrose, 2013).

However, in MCI patients exercise is seen as a way to prevent further general brain atrophy (Suzuki et al., 2013). From studies that did use a MCI population, exercising is recommended to reduce the risk for AD, or at least delay the progression to AD (D. E. Barnes, Whitmer, & Yaffe, 2007; Larson et al., 2006). As the ADL of MCI patients is not impaired, they are still able to live independent. That makes them possibly good candidates to participate in clinical prevention trails.

One of these clinical trails is the NeuroExercise study (Devenney, Sanders, Lawlor, Olde Rikkert, & Schneider, 2017). This study investigates the progression of MCI during a 12 month structured exercising program, consisting of both an aerobic and an anaerobic exercising program and a non-exercising control group.
The NeuroExercise study is a randomized control trial and included 42 participants that had an indication of a cognitive impairment, indicated by the criteria from Albert et al. (2011).

The NeuroExercise study has an anaerobic group to test what the influence of the social component of exercising in groups is on cognitive functioning in MCI patients. Anaerobic exercising is on a lower intensity, leaving out the physical effects expected from the aerobic exercise. The anaerobic group would then show more of the effects of the social component of exercising in groups.
Engaging in social activities could prevent the cognitive decline by increasing the cognitive reserve (Hughes, Flatt, Fu, Chang, & Ganguli, 2013). Cognitive reserve includes the ability to use different cognitive strategies. Cognitive reserve consists of IQ, age, education and occupation (Allegri et al., 2010). A larger cognitive reserve would mean that patients have more room to compensate before noticing a cognitive decline. IQ, age and education are difficult to modify in later life, so increasing social activity could increase the cognitive reserve.

Predicting the conversion of MCI to AD with hippocampal atrophy visualized with a MRI scan has been proven to be difficult (Schröder & Pantel, 2016). Neuropsychological tests are currently more sensitive to predict the progression from MCI to AD. However, findings from a MRI scan can be used as important clues as a loss of hippocampal volume is associated with a lower performance on specific neuropsychological tests (Peng et al., 2015).
One of the neuropsychological domains to test cognitive functioning might be temporal order memory (TOM), remembering the order of events. This is associated with the hippocampus and TOM declines faster in patients with MCI than in healthy agers (Devito & Eichenbaum, 2011; Fortin, Agster, & Eichenbaum, 2002; Galasko & Gilbert, 2013; Hsieh, Gruber, Jenkins, & Ranganath, 2014). This would suggest that a larger hippocampal volume would result in a better performance on the TOM task.

The underlying mechanism that possibly delays the onset of AD by exercising is neurogenesis, the growth and development of new neurons. Neurogenesis still happens in the adult brain and it particulary takes place in the dentate gyrus, a subfield of the hippocampus (Aimone, Deng, & Gage, 2014; Bergmann, Spalding, & Frisén, 2015).
According to MRI studies, brain atrophy in AD and especially atrophy in the hippocampus is substantial (Schröder & Pantel, 2016). This hippocampal atrophy is also seen in patients with MCI, but to a lesser extent than in AD patients. Compared to healthy elderly, a larger atrophy rate in the hippocampus has been found in MCI patients (Bergmann et al., 2015).
The finding that the atrophy rate in MCI patients is between the atrophy rate of healthy elderly and elderly with AD, contributes to the current idea that MCI might be a pre-AD state.

Neurogenesis might lead to an increase in hippocampal volume and therefore delay the hippocampal atrophy that is present in MCI patients. In healthy older adults, one year of aerobic exercise led to an increase in hippocampal volume. Exercising served as a protective factor against hippocampal volume loss, as the control group experienced a decline in their hippocampal volume (K. I. Erickson et al., 2011; Kirk I. Erickson et al., 2009).
A recent review study showed that in a heterogenic population (healthy elderly, MCI patients and schizophrenic patients) exercising prevented the decline in hippocampal volume. Researchers found that hippocampal volume remained stable instead of actually increasing due to exercising (Firth et al., 2018).

The above mentioned studies focus however mostly on healthy elderly, which leaves the effect of exercise on hippocampal volume in MCI patients unclear. To our current knowledge, no studies have been investigating the influence of exercising on hippocampal volume in a MCI population.
It thus remains unclear whether exercising after the diagnosis of MCI can still delay the progression to AD through an increase of hippocampal volume.

In the current study, the primary aim is to determine the relation between exercising (aerobic or anaerobic) and non-exercising and hippocampal volume. Two times during the NeuroExercise study a MRI scan was made. The MRI-scan was made at the beginning of the study (T0) and after 6 months (T1). These scans were analysed to determine hippocampal and dentate gyrus volume. As exercising should prevent a loss of hippocampal volume (K. I. Erickson et al., 2011), it is expected to find a difference between the exercising groups and non-exercising group.

The secondary aims are to look at the effect of exercise on TOM, memory scores from the MoCA and the general MoCA score in relation to hippocampal volume.
As TOM is related to the hippocampus, it is expected to find a better performance on the TOM task when a larger hippocampal volume is found.
The relation between the MoCA score and hippocampal volume will also be determined, as memory scores from the MoCA are possibly related to hippocampal volume (Ritter, Hawley, Banks, & Miller, 2017).

# Method

## General

A total of 42 participants were included in the NeuroExercise study. Participants were recruited through the memory clinic or local newspapers. The full inclusion criteria of the NeuroExercise study can be found in appendix A (Devenney et al., 2017).
The MRI was an additional measurement for which the participants had to fill in an additional informed consent before the MRI scan was performed. For example, participants were excluded from the MRI scan if they were suffering from claustrophobia or had metal objects which could not be removed.
19 participants completed their MRI at the beginning (0 months) and halfway the study (6 months). Of those 19 participants, 2 scans had to be left out of the analysis, resulting in 17 participants in total. Of the other 23 participants, no MRI was made of 14 participants, 5 participants dropped out and 4 participants were not able to make their second MRI due to their medical condition.

The 17 participants had a mean age (in years) of 71 with a standard deviation (SD) of (± 7,4). 6 participants were in the aerobic group, 7 participants were in the anaerobic group and 4 participants were in the control group. 12 participants were male and 5 participants were female. The mean MoCA score, indicating the severity of the MCI at T0 was 22,9 (± 3,1) and at T1 was 22,2 (± 4,8).

## Procedure

### Exercising

After inclusion in the Neuro Exercise study, participants were placed in an aerobic, anaerobic or non-exercising control group. Examples of these classes for the aerobic group are boot camp, sports and games and running/Nordic walking. For the anaerobic group the classes consist of walking, stretching and toning and yoga. Guidelines were set up for the heart rate and BORG score for both the aerobic and anaerobic group, to check if the participants are training according to their program. These guidelines can be found in the study of Devenney et al. (2017).
During the exercise program, participants in the exercise groups were visited by someone from the NeuroExercise team during their exercise class once a month. During these visits their heart rate was monitored with a Garmin Vivosmart and the participants rated the intensity of their exercise at the end of the class according to the Borg scale, see appendix C. These data were used to check if the above mentioned guidelines were met.
The Borg scale was noted after every class by the participants themselves and after the monthly visits by someone from the NeuroExercise team as well.

At the beginning of the study and after six months, the participants wore an Actiwatch (Philips respironics, Actiwatch 2) for seven consecutive days. The data of the Actiwatch is used to check whether the participants were exercising according to their program. Furthermore, the Actiwatch monitors the activity level of all participants, also the control group.
The Actiwatch data was converted in Matlab and the mean of the counts per minute (CPM) were calculated for the 7 consecutive days the Actiwatch was worn. The differences in the mean values at T1 and T0 were used to see whether the participants were more or less active compared to the baseline measurement.
After the Actiwatch was worn for a week, a subjective questionnaire was also taken. This questionnaire, the LAPAQ (LASA physical activity questionnaire), combines questions about exercising and ADL activities. A number is than generated where a higher number indicates a higher activity level.

### MRI acquisition

MRI acquisition was performed with a 3-T MRI scanner (Siemens Magnetom Prisma syngo MR D13D, The Donders Institute, Nijmegen), using a 32 channel array coil. 3-D T1-weighted MRI gradient- echo MP2RAGE sequence was obtained with the following scanning parameters: 0.8 mm thickness; 256 slices; scanning time 12:30 min; repetition time/echo time 5500/3.28 ms; number of signal averages 1; matrix 256x320x320; field of view 256x256 mm; inversion times 700 ms and 1100 ms; and flip angle 6°. The final voxel size was 0.8x0.8x0.8 mm (x*y*z). The MP2RAGE sequence is obtained by combining the two MPRAGE images. This leads to multiple benefits for the image (Marques et al., 2010).

## Data analysis

### FreeSurfer

The scans were analyzed with FreeSurfer v6.0.0. A detailed guide of how to install and use FreeSurfer can be found in appendix B. This guide was written during and after the setup of FreeSurfer and specified on the used MRI data from the NeuroExercise study.

As part of the general analysis, FreeSurfer performed an automatic sub cortical segmentation of the brain, which means the brain will be divided in different brain structures (e.g. white and gray matter, intracranial volume, etc.) and their volumes were calculated. The volumes were all estimated in $mm^3$. After the general analysis, these volumes were automatically saved in a text file.

The data from the general analysis was used for a second analysis, together with the T2 image. This analysis was the hippocampal subfields analysis and looked at the hippocampus with more detail. After the hippocampal subfields analysis, estimated volumes of the total hippocampus and its subfields (CA1, CA3, CA4, Dentate Gyrus, hippocampal tail) were generated and automatically saved in a text file. These volumes were also given in $mm^3$. The hippocampus with its different subfields is visualized in figure 1.

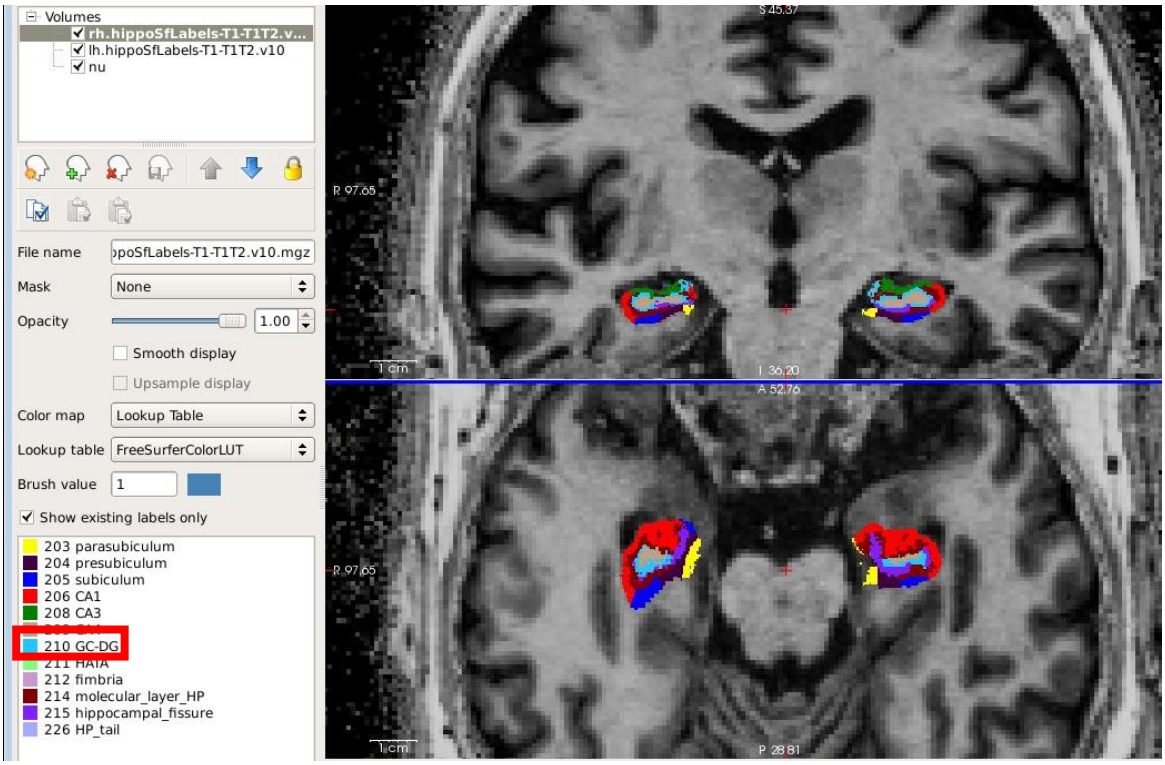


**Figure 1** Outcome after the hippocampal subfields analysis. The full hippocampus is shown with its subfields. The light blue colour represents the dentate gyrus (indicated with the red bracket on the left side of the image).

From each scan of the participants the T1 and T2 images were used for the general analysis. T1 and T2 MR images result from a different relaxation time during the scan. This makes the different structures visible on the MR images.

For the general analysis, FreeSurfer applied at first a skull strip to remove the skull and meninges from the image. The removal of the skull and meninges was done with a hybrid approach, described in Ségonne et al. (2004). Ségonne et al. writes that segmentation of the different structures was done automatically and based on probabilistic information. This information was automatically estimated from a training set which was manually labelled and has shown to be comparable in accuracy to manual labelling. Furthermore it is of sufficient sensitivity to detect changes in the volumes of non-cortical structures (e.g. hippocampus), which is useful for the detection of changes in the onset of MCI (Fischl et al., 2002).

The FreeSurfer v.6.0.0 construction of the anatomical atlas of the hippocampal subfields is based on ex- and in-vivo data (Iglesias et al., 2015). The ex-vivo data was scanned with an extraordinary resolution to make an accurate segmentation of the subfields. The in-vivo data was used to discriminate between other neighbouring structures, which was done manually. The combination of the ex- and in-vivo data resulted in an atlas that has a larger accuracy than in the previous hippocampal subfields analysis.

After the hippocampal subfields analysis and the general analysis of Freesurfer, results were checked manually to ensure the validity of the data (Tae, Kim, Lee, Nam, & Kim, 2008; Wenger et al., 2014). Previous studies found that the hippocampus with the amygdale border and the hippocampal tail with the lateral ventricle border are often incorrect. This leads to an overestimation of the hippocampal volume. Therefore the data was checked for this overestimation and corrected. Volumes were left out of the analysis when the deviation was larger than two times the standard deviation below or above the mean value.

## Statistical analysis

A multiple regression model was used to test the data. The outcome parameter was the change in hippocampal volume and dentate gyrus volume (both in $mm^3$) after 6 months of exercising. To account for the intensity of exercising, the exercise groups (aerobic, anaerobic or control) were taken in the analysis, as well as the Actiwatch data, LAPAQ score and attendance at the exercise classes. When results indicate a significance below the $p = 0{,}05$ value, it was assumed a significant difference was found.

The Actiwatch data was used as an objective measurement that reflected on the intensity (in CPM) of the activities of daily living and exercising, as the Actiwatch registered movements for 7 consecutive days. The mean CPM was calculated for the first half year, as well as the difference in CPM compared to the baseline measure (T0) to see if they were more or less active after 6 months.
The LAPAQ had a similar outcome, but it is a subjective questionnaire about the activities of daily living regarding the past two weeks. The outcome was given in numbers, where a higher activity level resulted in a higher score.
The attendance at the exercise classes was a subjective measure as well, but gave an estimation of the attendance of the past half year. From this point of view it was expected that a higher attendance rate might result in a larger change of hippocampal volume.

# Results

The data of 17 participants with a mean age in years of 71, were used in this study. All the participants were diagnosed with aMCI and 29% (5 participants) were female. In table 1, descriptives of the participants are found for their left and right hippocampus and dentate gyrus.
Scans that were more than two times the standard deviation below or above the mean value of the hippocampus and dentate gyrus, were left out of the analysis. The tables where the values and cut off scores are in, can be found in appendix D, table 10 and 11. According to the standard deviation calculated by SPSS, one participant had to be left out. The analysis was thus done with 17 participants in total.

**Table 1** Values of the participants for the volumes of the left and right hippocampus and dentate gyrus at T0 (baseline) and at T1. Volumes are all given in $mm^3$.

| **Structure** | **Value (mean ± SD)** |
|---|---|
| Left hippocampus (T0) | 2852 ± 305,6 |
| Left hippocampus (T1) | 2943 ± 329,4 |
| Right hippocampus (T0) | 3045 ± 380,8 |
| Right hippocampus (T1) | 3101 ± 389,1 |
| Left dentate gyrus (T0) | 221 ± 27,2 |
| Left dentate gyrus (T1) | 232 ± 30,6 |
| Right dentate gyrus (T0) | 241 ± 35,3 |
| Right dentate gyrus (T1) | 246 ± 37,5 |

The full table from SPSS with its values of the hippocampus and dentate gyrus can be found in table 4, appendix D.

In table 2, the descriptive statistics of the difference in volume after 6 months (Δ volume) of exercising are given for the left hippocampus (LH), right hippocampus (RH), left dentate gyrus (LDG) and right dentate gyrus (RDG).

**Table 2** The values of the difference in volumes between 0 months and 6 months of exercising. Volumes are given in $mm^3$.

| **Structure** | **Value (mean ± SD)** |
|---|---|
| Δ Left hippocampus | 79,1 ± 216,0 |
| Δ Right hippocampus | 55,1 ± 356,1 |
| Δ Left dentate gyrus | 11,2 ± 27,5 |
| Δ Right dentate gyrus | 4,9 ± 37,0 |

The full table of the change in volume can be found in table 5, appendix D.

The values from table 2 are visualized in box plots. As can be seen in figure 3 and 5, the right side seems to increase most in volume for both the hippocampus and dentate gyrus in the aerobic group and to decrease for the hippocampal and dentate gyrus volume in the control group. The left side shows a larger variance within the groups for the hippocampus and dentate gyrus, by showing an increase and decrease in volume in all the groups.

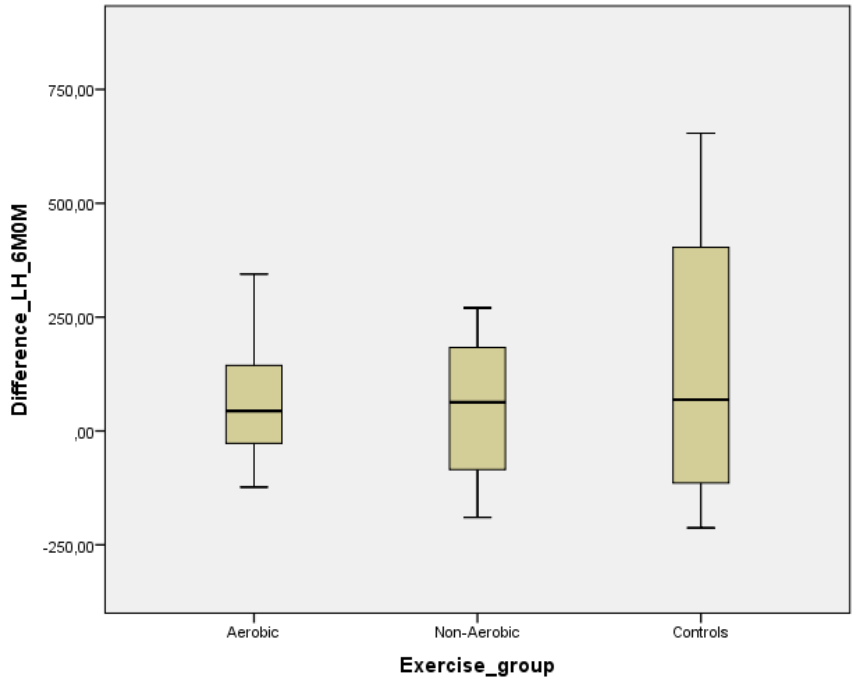


**Figure 2** A box plot of the change in volume (in $mm^3$) in the left hippocampus after six months (Δ volume).

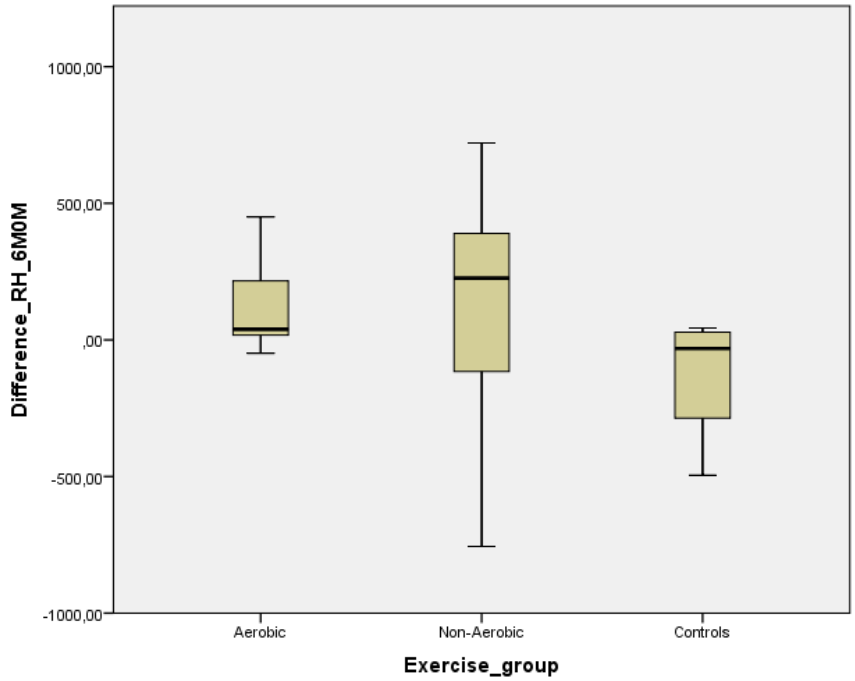


**Figure 3** A box plot of the change of volume (in $mm^3$) in the right hippocampus after 6 months (Δ volume).

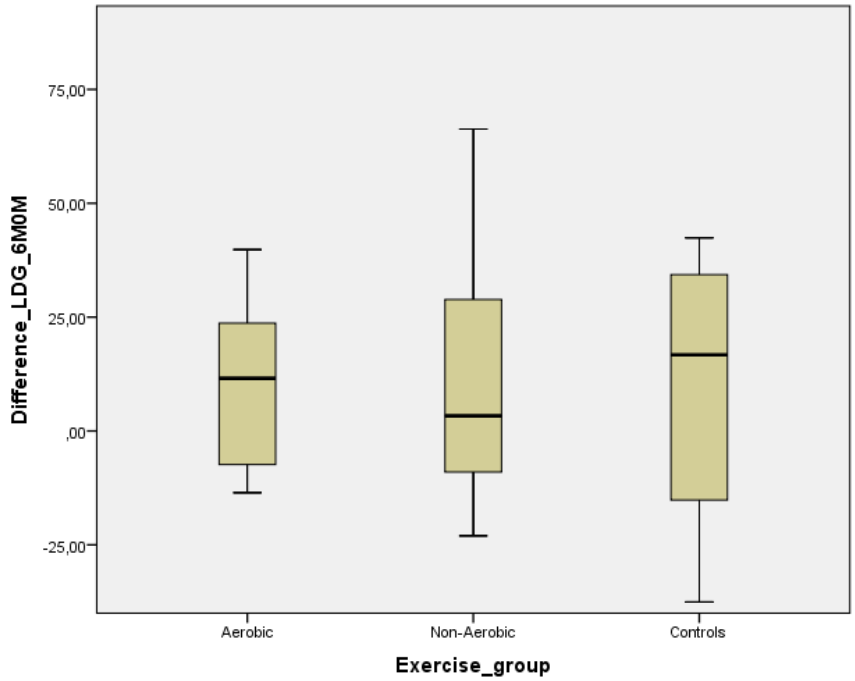


**Figure 4** A box plot of the change of volume (in $mm^3$) in the left dentate gyrus after 6 months (Δ volume).

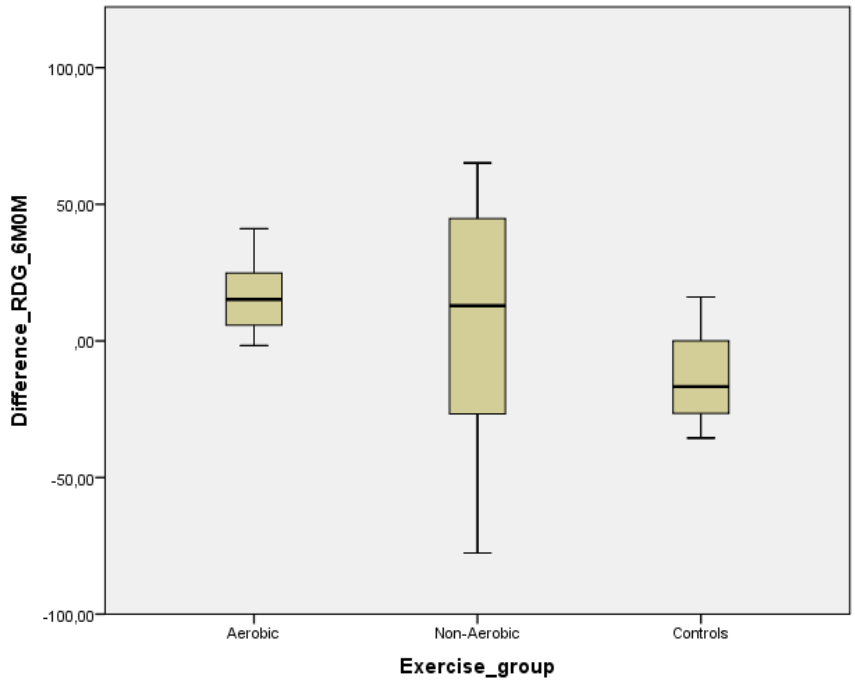


**Figure 5** A box plot of the change of volume (in $mm^3$) in the right dentate gyrus after 6 months (Δ volume).

In figure 6 to 9 is visualized with a scatter plot if the amount of exercise classes attended in the first half year of the study had an influence on the change in volume in left and right hippocampus and dentate gyrus. Only the exercise groups (aerobic or anaerobic) were used, because the control group did not follow the exercise intervention. The correlation values ($R^2$) for the change in left and right hippocampal volume and left dentate gyrus volume with the amount of exercise classes are below $< 0{,}4$ which makes the correlation very weak and not interesting to take into account. For the right dentate gyrus the $R^2 = 0{,}5$, which is more interesting to look at. However, in figure 7 and 9 is

visualized that the participants who attended more than a 100 exercise classes in 6 months show a decrease in right hippocampal and dentate gyrus volume.

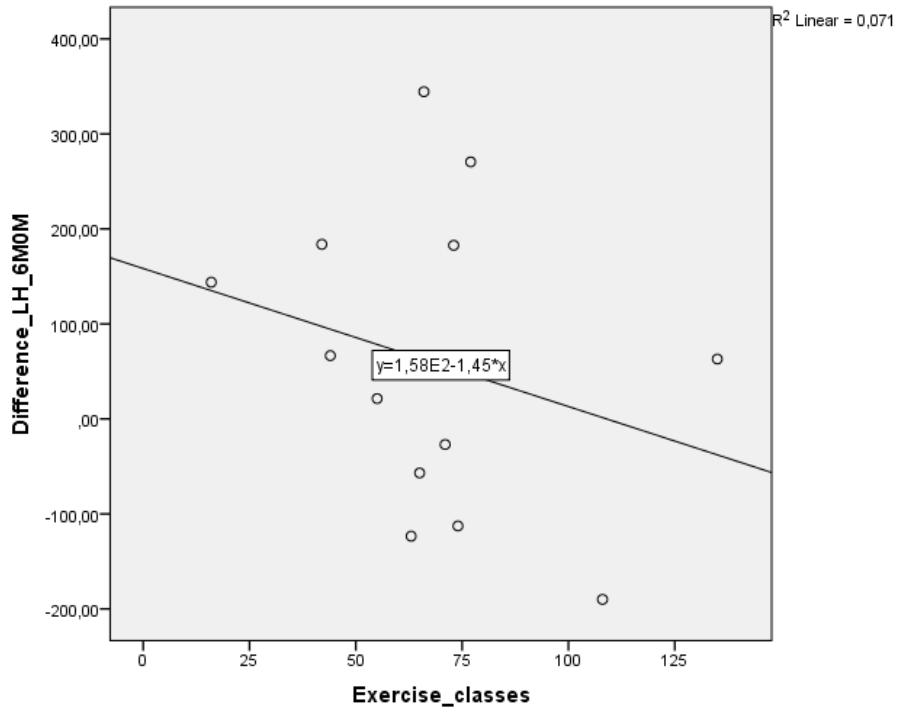


**Figure 6** Scatter plot of the amount of exercises for the first half year of the study plotted against the difference in left hippocampal volume (in $\boldsymbol{mm^3}$) after 6 months (Δ volume).

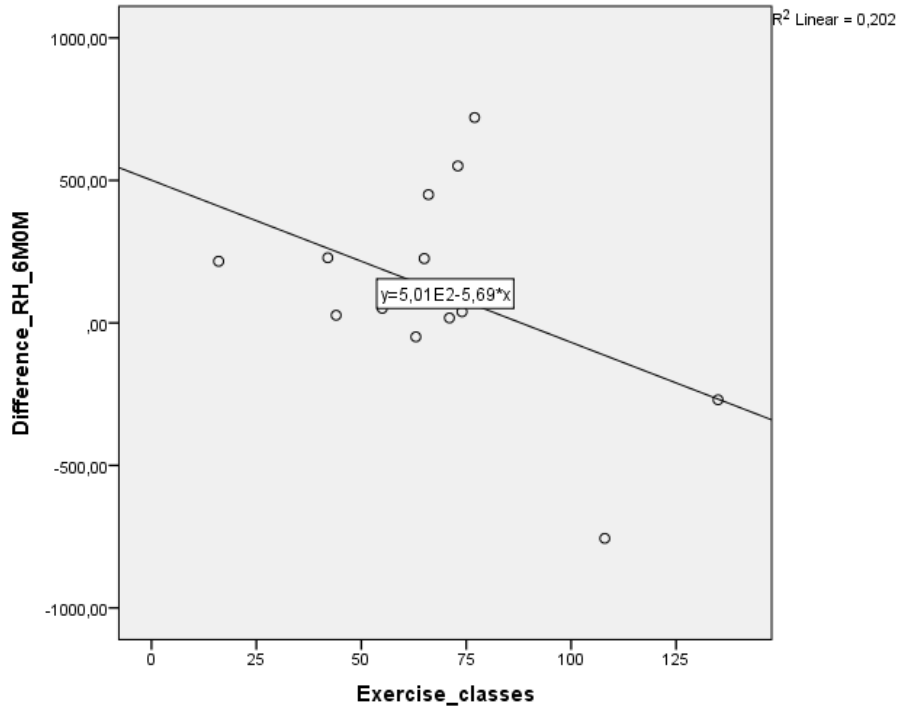


**Figure 7** Scatter plot of the amount of exercises for the first half year of the study plotted against the difference in right hippocampal volume (in $\boldsymbol{mm^3}$) after 6 months (Δ volume).

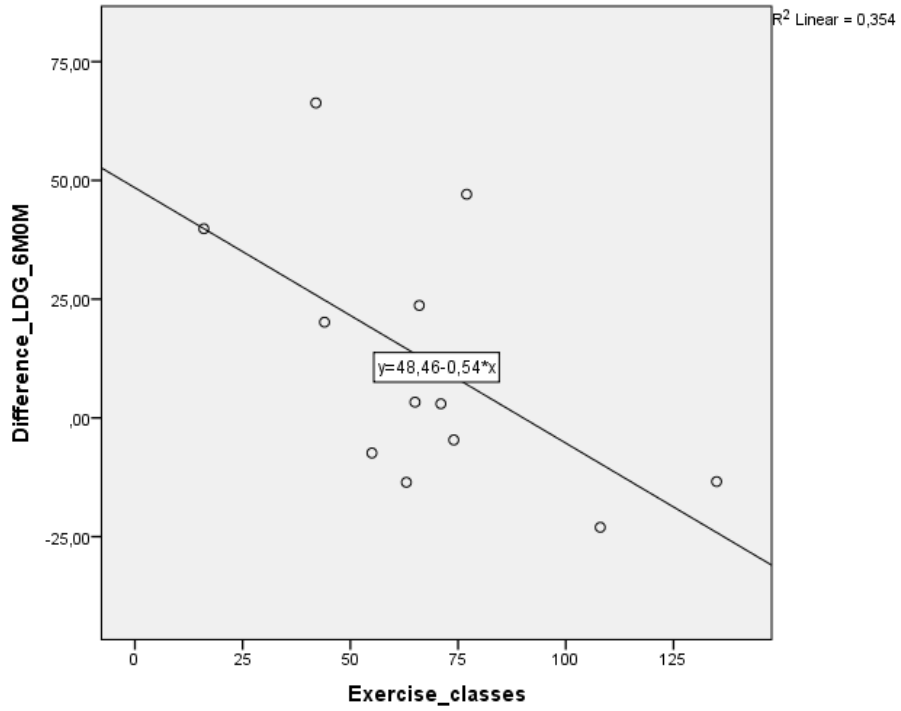


**Figure 8** Scatter plot of the amount of exercises for the first half year of the study plotted against the difference in left dentate gyrus volume (in $\boldsymbol{mm^3}$) after 6 months (Δ volume).

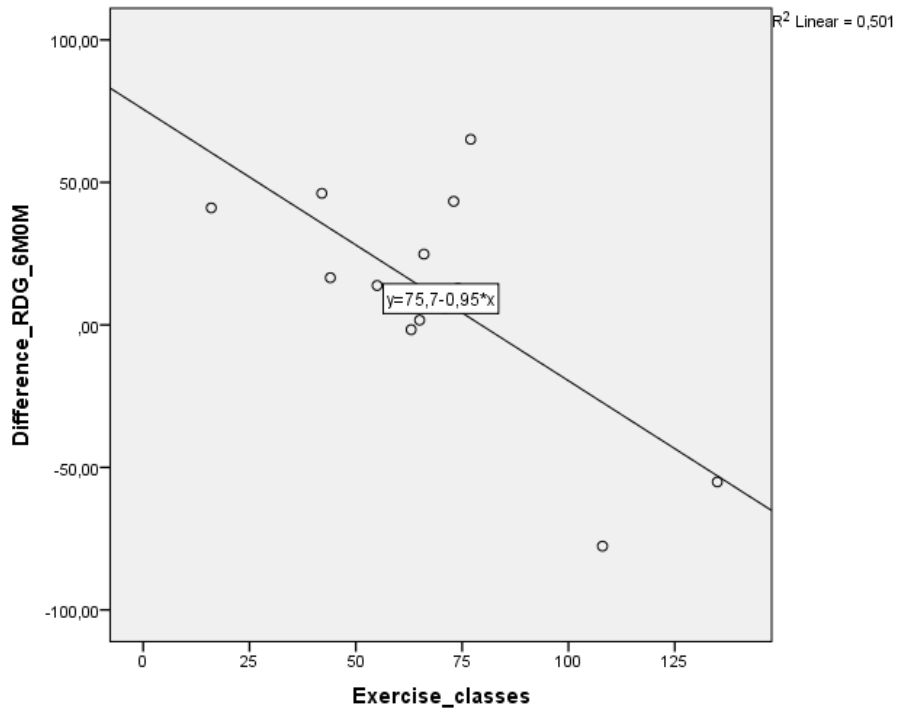


**Figure 9** Scatter plot of the amount of exercises for the first half year of the study plotted against the difference in right dentate gyrus volume (in $\boldsymbol{mm^3}$) after 6 months (Δ volume).

In figure 10 to 13 the relation between the difference in CPM and left and right hippocampal and dentate gyrus volumes are visualized. The correlation between the CPM and volumes are all very low, $R^2 < 0{,}05$. Furthermore, it only seems that being more active after 6 months compared to the baseline measurement, is beneficial for the dentate gyrus, as those figures (12 and 13) show an increasing line.

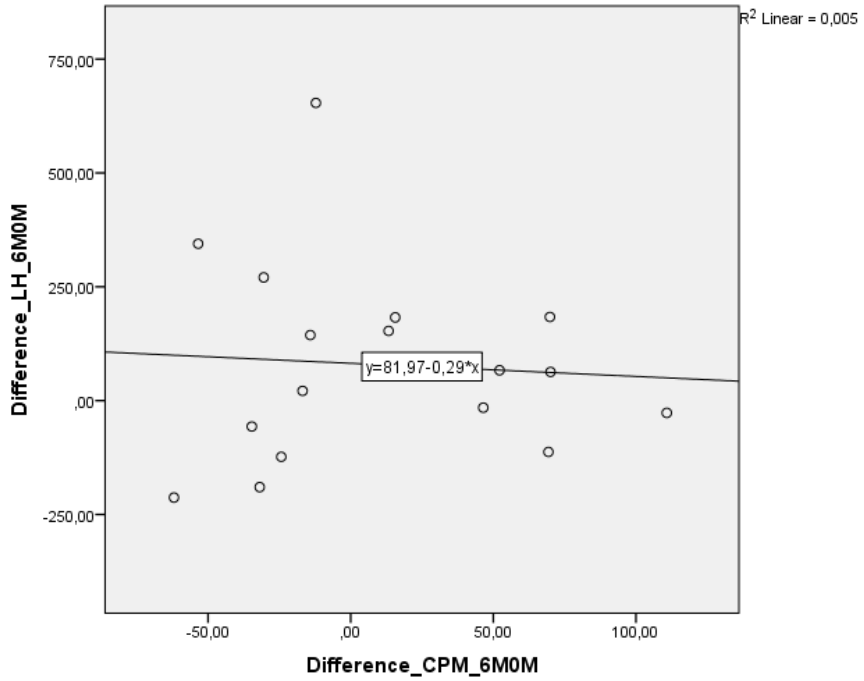


**Figure 10** Scatter plot of the relation between the change in counts per minute (CPM) and the change in left hippocampal volume (in $\boldsymbol{mm^3}$) after 6 months.

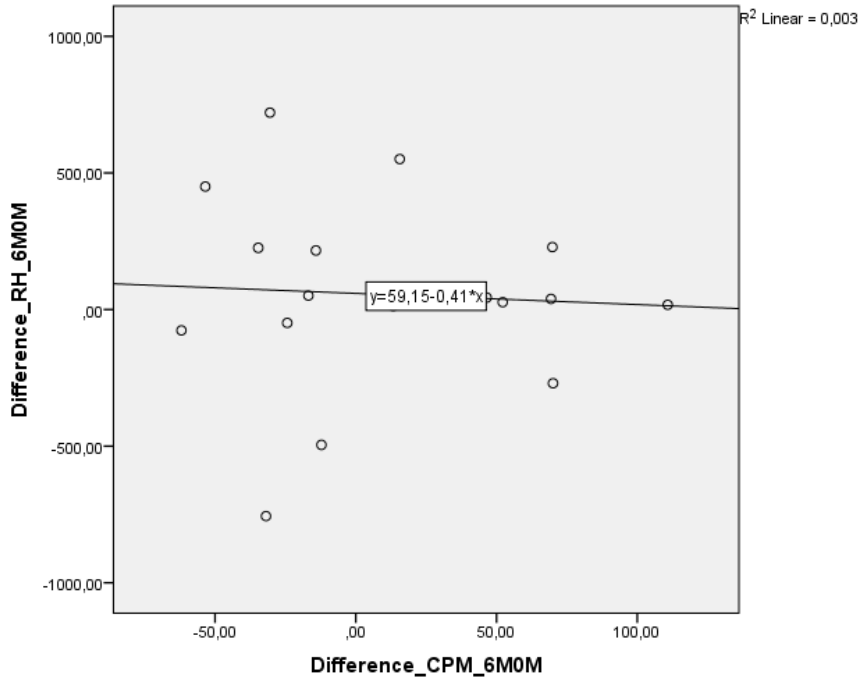


**Figure 11** Scatter plot of the relation between the change in counts per minute (CPM) and the change in right hippocampal volume (in $\boldsymbol{mm^3}$) after 6 months.

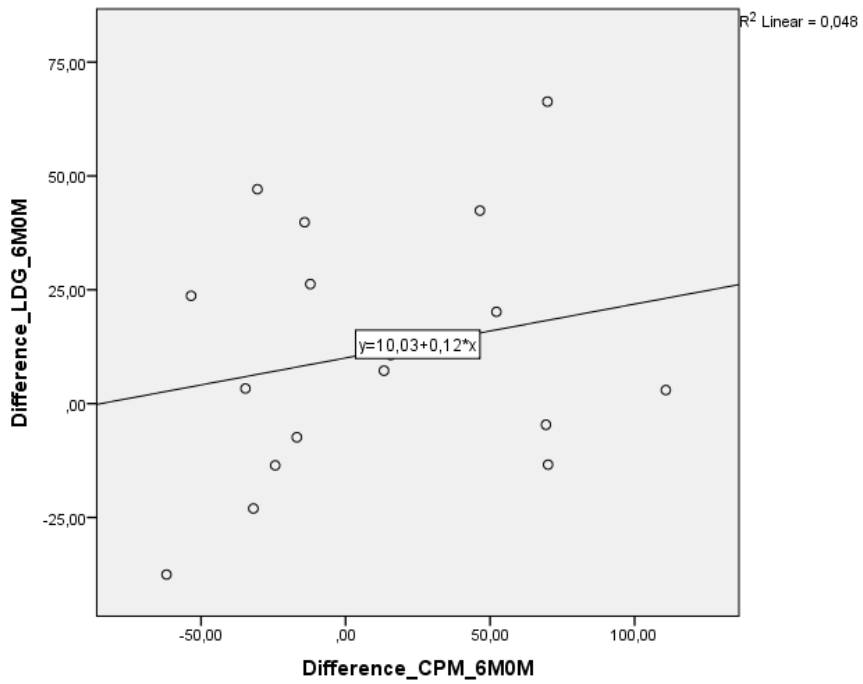


**Figure 12** Scatter plot of the relation between the change in counts per minute (CPM) and the change in left dentate gyrus volume (in $mm^3$) after 6 months (Δ volume).

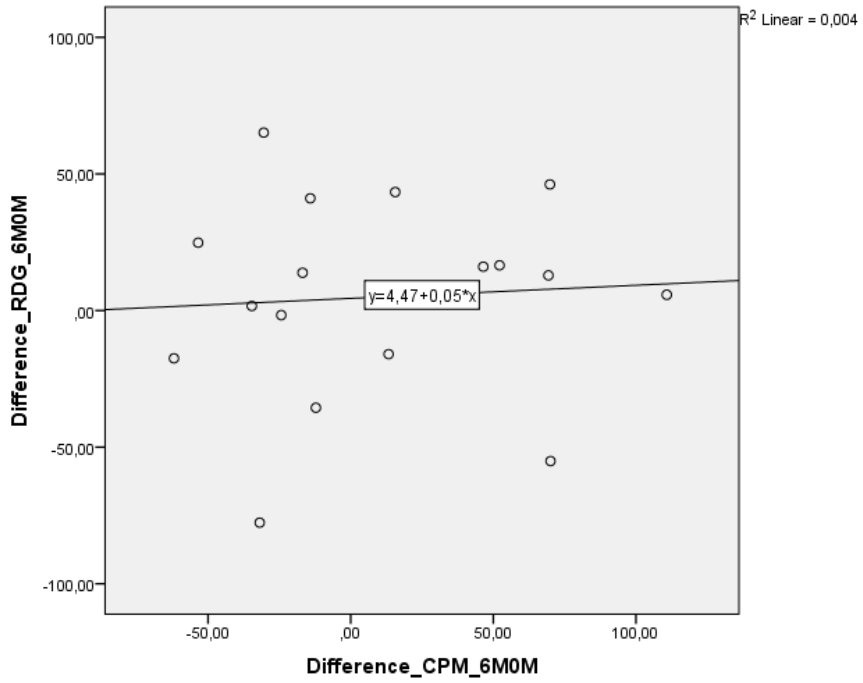


**Figure 13** Scatter plot of the relation between the change in counts per minute (CPM) and the change in right dentate gyrus volume (in $mm^3$) after 6 months (Δ volume).

The B-value with its standard error (SE) and the p-values of the multiple regression are given in table 3 for each dependent and independent variable. The full analysis can be found in appendix D, tables 6 to 9. As can be seen, the scores from the LAPAQ were left out of the analysis due to large differences in scores within and between participants.

**Table 3** The values of the multiple regression analysis of the different dependent variables with the independent variables. The dependent variables are the change in volume after 6 months of exercising (Δ volume). SE is the standard error of B.

| | **Dependent variables** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Independent variables** | Δ Left hippocampus | | | Δ Right hippocampus | | | Δ Left dentate gyrus | | | Δ Right dentate gyrus | | |
| | **B** | **SE** | **p** | **B** | **SE** | **p** | **B** | **SE** | **p** | **B** | **SE** | **p** |
| Exercise group | 28.450 | 82.148 | 0.735 | -132.267 | 140.769 | 0.366 | -2.840 | 9.501 | 0.770 | -21.348 | 13.219 | 0.132 |
| Amount of exercise classes | -1.008 | 1.625 | 0.547 | -1.357 | 2.785 | 0.635 | -0.260 | 0.188 | 0.191 | -0.395 | 0.262 | 0.157 |
| Mean CPM | -0.882 | 0.666 | 0.210 | -0.619 | 1.141 | 0.597 | -0.117 | 0.077 | 0.153 | -0.069 | 0.107 | 0.532 |
| Difference in CPM | -0.713 | 1.216 | 0.569 | -0.795 | 2.083 | 0.709 | 0.072 | 0.141 | 0.620 | 0.031 | 0.196 | 0.876 |

As can be seen in table 3, there doesn't seem to be an influence of the independent variables on the dependent variables. None of the p-values are close or below the set significance level of $p < 0,05$.

However, when looking at the relation between the baseline hippocampal volume and age (figure 14 and 15), it is visualized that the correlation is very weak ($R^2 = 0{,}271$ for the left hippocampus and $R^2 = 0{,}040$ for the right hippocampus). It is therefore difficult to interpret the values in table 3, as older age would result in a lower hippocampal volume and a stronger correlation was expected.

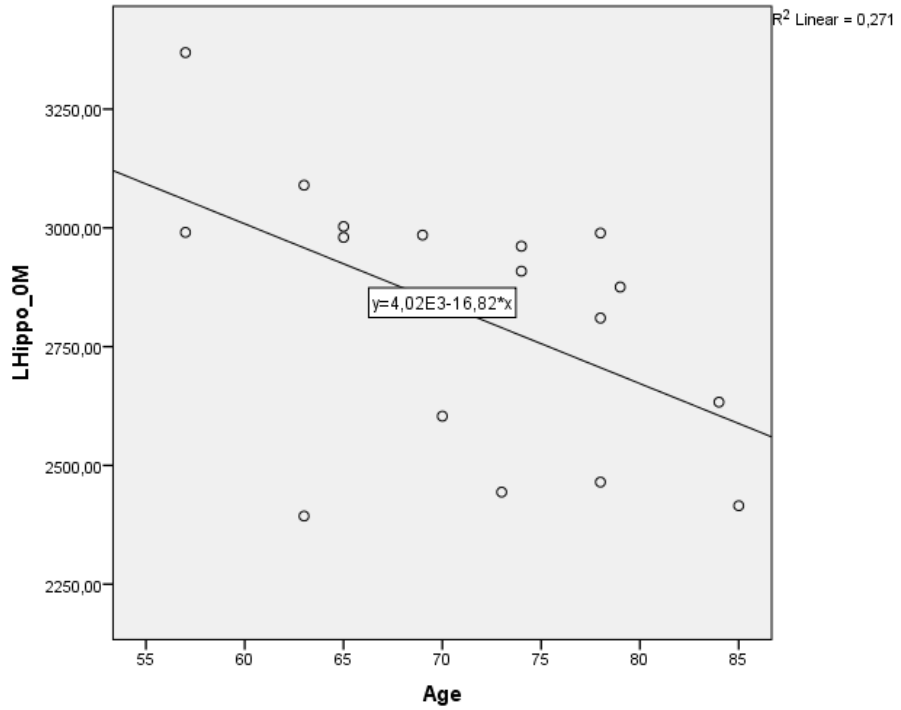


**Figure 14** Scatterplot of the relation between age (in years) and left hippocampal volume (in $\boldsymbol{mm^3}$) at the beginning of the program (T0).

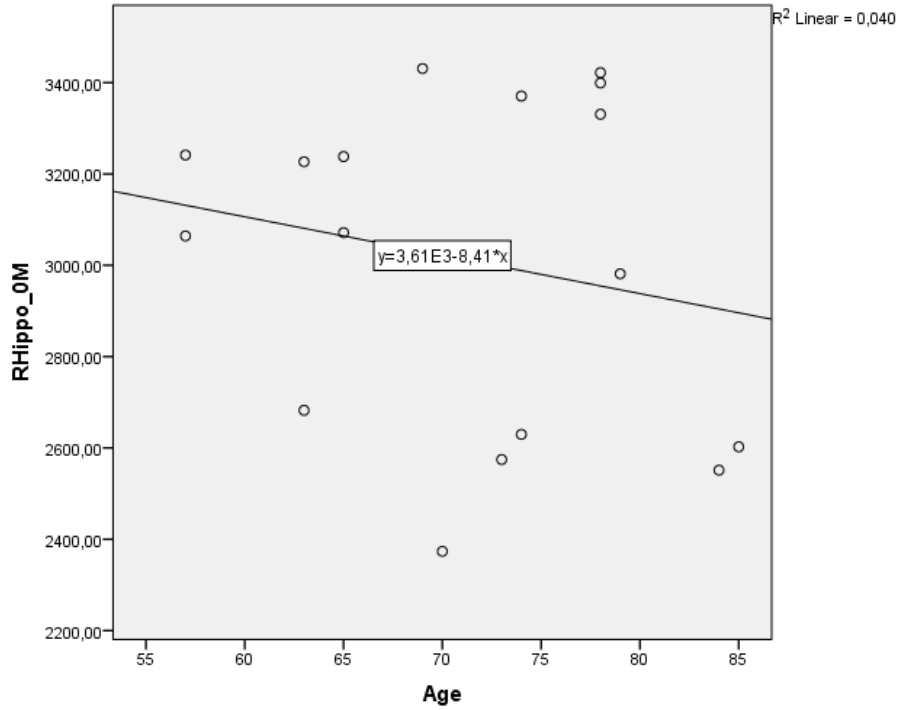


**Figure 15** Scatterplot of the relation between age (in years) and right hippocampus volume (in $\boldsymbol{mm^3}$) at the beginning of the program (T0).

# Discussion

This study was set up to look at the influence of exercising on hippocampal volume in elderly with MCI. Literature is mostly focused on healthy elderly and although results are very promising for the influence of exercising on hippocampal volume in that population, it remains unclear whether the same effect can be found in a MCI population.
17 participants from the NeuroExercise study completed a MRI scan at the beginning of the program and after 6 months in the program. Hippocampal volume and dentate gyrus volume were measured from these scans. The difference in these volumes after 6 months (Δ volume), were correlated with their activity level in CPM, amount of exercise classes and exercise group they were placed in according to the program. A multiple regression showed the relation of the Δ volumes with the independent variables.

The results from the multiple regression analysis show high p values ($p > 0{,}15$). This would indicate that none of the independent variables have an effect on the dependent variables. This might be due to different factors.
The hypothesis was based on data from healthy elderly (K. I. Erickson et al., 2011; Kirk I. Erickson et al., 2009; Firth et al., 2018), which is different compared to a MCI population. The MCI population is very heterogenic as MCI might be temporary, they can go back to their normal level of cognitive functioning. The chance of staying in the MCI state is also present but they can also decline further to AD (Smith & Bondi, 2013, p. 74). Thus in a small group of 17 participants the variance within the MCI state can be large. However, this heterogeneity cannot be determined from factors like age and gender, but by following the participants for a longer period. Therefore it is not possible to determine the heterogeneity in this group during this study.

Furthermore, when looking at the relation between age and hippocampal volume at the baseline MRI (T0) (figures 14 and 15), it is seen that the correlation is very low ($R^2 = 0{,}271$ for the left hippocampus and $R^2 = 0{,}04$ for the right hippocampus). This was unexpected as a higher age results in a lower hippocampal volume and a stronger correlation was expected.
In the study of Bergmann et al. (2015) it is suggested that there is hippocampal atrophy in healthy elderly, but the atrophy rate is larger in patients with MCI. Combining the findings of Smith & Bondi (2013) and Bergmann et al. (2015), these figures might be explained by the fact that within the MCI state atrophy rates can variate, as the progression of the MCI is variable as well. This would lead to the suggestion that patients who have a temporary MCI state are more close to healthy elderly and probably have a lower atrophy rate than MCI patients who are declining further to AD. This would also explain the normal distribution of the data, as the data is not disturbed normally. Thus this suggested larger variance in atrophy rate might explain why the relation between age and hippocampal volume is very weak and why the effect of exercising on hippocampal and dentate gyrus volume is still unclear.

To take a closer look at the independent variables and the dependent variables, box plots are made were the exercise groups and the Δ volumes are visualized (figure 2 to 5). In figure 3 the Δ right hippocampal volume is plotted and in figure 5 the Δ right dentate gyrus volume. In both these figures the aerobic group seems to have an increase in their hippocampal and dentate gyrus volume and the control group shows a decrease in the volumes. These results thus suggest that aerobic exercise leads to an increase for the volumes of the hippocampus and dentate gyrus on the right side, whereas the left side (both the hippocampus and dentate gyrus) show a large variance for increasing and decreasing for all the groups. However, this is based on the groups the participants were placed in for the study and do not reflect on their activity level. It is therefore difficult to state that aerobic exercising leads to an increase in the volumes of the right hippocampus and right dentate gyrus. Finding different results for the left and right side of the brain has been reported previously (Firth et

al., 2018). The review study of Firth et al. (2018) shows that some studies report an increase on left side while others find an increase on the right side after exercising. These findings are thus still heterogenic.

A way to reflect more on the activity levels of the participants is to determine the relation between the amount of exercise classes they attended in half a year with the Δ volume. For this analyses only the exercise groups (aerobic group and anaerobic group) were taken into account.
A scatterplot was made to determine this relation, see figure 6 to 9. It is very interesting that again, the Δ right hippocampus and especially the Δ right dentate gyrus shows the most progress, when exercising below the threshold of a 100 classes per half year. In this study, the participants exercised with a maximum of one exercise class a day. With these results there must be accounted for that it is only saying something about the amount of exercise classes attended, and not about the intensity or duration of the exercise. Participants who are more structured, might follow more exercise classes compared to participants who attended less exercise classes. However, this gives no insight on the intensity.
Looking at these results, it thus seems that attending more than a 100 exercise classes in half a year shows a decrease in hippocampal volume and dentate gyrus volume. When participants stayed below the threshold of a 100 exercise classes per half year, data shows a maintenance or even an increase in hippocampal and dentate gyrus volumes.
This was not expected, as more exercising would stimulate neurogenesis and lead to an increase of volumes (Aimone, Deng, & Gage, 2014; Bergmann, Spalding, & Frisén, 2015). However, 6 months is a short period of time. Although neurogenesis can happen within that time frame, it might be that the neurogenesis in these 6 months is not enough to compensate for the atrophy that is present in MCI patients.

The CPM from the Actiwatch data gives an insight on the activity of all the participants, including the participants from the control group. These data give an insight in the overall activity of all the participants during the week they wore an Actiwatch. The Actiwatch was worn for a week at T0 and T1. The difference in mean CPM of these weeks were calculated to see whether they were more active at T1 compared to the baseline measurement (T0). In figure 10 to 13 the difference in CPM after half a year is plotted against the Δ volumes of the hippocampus and dentate gyrus. Only for the dentate gyrus it seems that being more active has a positive influence on the volumes, by showing an increasing line.
From these graphs (figure 10 to 13), no clear distinction can be made between left and right side but hippocampal volume does not seems to benefit as much from being more active after half a year as the dentate gyrus does.
This might be explained by the fact that neurogenesis takes place in the dentate gyrus and that after half a year of being more active, new neurons are made. This is however a very short period of time and might therefore not have an influence on total hippocampal volume yet.
For example, the studies of Erickson et al. (2009, 2011) were done with healthy elderly and based on one year of aerobic exercising. MCI patients might therefore need an even longer intervention to see an effect of neurogenesis on the total hippocampus volume. When looking at the participants from this study, the atrophy is still expected. However, the time frame of 6 months is very limited and showing a less decrease or even an increase in volumes makes the suggestion that exercising helps.

Other factors that might influence the current found results is the software that has been used to analyse the volumes of the hippocampus and dentate gyrus. FreeSurfer is known to overestimate the volumes (Tae et al., 2008; Wenger et al., 2014). However, estimating volumes with an automatic segmentation is very difficult and the data where FreeSurfer is based on, shows good accuracy and sufficient sensitivity to detect changes in non-cortical structures (Fischl et al., 2002; Ségonne et al. 2004).

## Limitations

A few secondary aims were mentioned in the introduction but unfortunately had to be left out. This was due to the current found results, missing data and limited time.
Furthermore, the sample size was small (17 participants, 6 in the aerobic group, 7 in the anaerobic group and 4 in the control group) and as previously mentioned, the MCI population is very heterogenic. It is therefore very reasonable to assume that this sample size does not reflect well enough on the MCI population to find statistical differences.
The exercise classes the aerobic and anaerobic group were attending, were widespread in activities participants were doing. This results in a large variance of exercises within groups and the question whether some exercises might be more beneficial than others.
FreeSurfer shows good sensitivity in detecting changes in non-cortical structures (Fischl et al., 2002). However, it has not been used much in studies and might therefore have also influenced these results.
The LAPAQ was used as a subjective measure for the activity level of all the participants. However, this questionnaire is very precise so it is open to wide interpretation. This resulted in data that could not be used in the statistical analysis.

## Recommendations and future work

The influence of exercising on hippocampal volume in a MCI population has hardly been studied and therefore it might be useful to set up a larger cohort of participants and/or exercise for a longer period (e.g. 12 months or longer). This could give a better insight on the influence of exercising on hippocampal volume in a MCI population.
As mentioned, in this study there were no standardized exercise programs. It might be useful to set up a program that each participants has to follow, to minimize the differences of intensity in classes within groups.
In the current study, activity levels were measured by an Actiwatch which was worn for 7 consecutive days. The Actiwatch is a wrist worn device and thus reacts on the amount of movements the arm makes. To get a better view of activity levels, using a different device is something to consider. Also, when measuring the activity levels with a subjective questionnaire (e.g. LAPAQ) the questionnaire should be more standardized to minimize the difference between the assessors.

To analyse the volumes of the hippocampus and the subfields, FreeSurfer was used in this study. Although FreeSurfer is still getting updates and improving on the accuracy in their analyses, manually labelling is still more accurate. Before reproducing a similar study, it should be considered which program to use for analysing the MR images and if there is chosen to do this manually or use software that has an automatic based segmentation.

# Conclusion

From the results in this study, the influence of exercising in elderly participants with MCI on hippocampal volume remains unclear. The MCI population is very heterogenic, the sample size was small and 6 months of exercising might not be enough to detect an increase or decrease in hippocampal volume.

Although interpreted with caution, it can be concluded that the amount of exercises, when below the border of 100 exercises per half year, leads to an increase in volume for the right hippocampus and dentate gyrus. When looking at the activity level, a higher activity level at 6 months, compared to baseline, seems to increase the dentate gyrus volumes but does not have an effect yet on total hippocampal volume. This might be due to the fact that the neurogenesis for the 6 month period is too short to overcome the atrophy that is present in the MCI population.
However, further research will be needed to confirm these results.

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# Appendices

## Appendix A: In- and exclusion criteria

Inclusion criteria from the Neuro Exercise study (Devenney et al., 2017)

**INCLUSION**
Participants with the following criteria were eligible to participate:
(1) MoCA score 18–26
(2) stable medical condition for more than 6 months
(3) stable medication for more than 3 months
(4) adequate visual and auditory acuity to complete neuropsychological testing
(5) electrocardiogram without significant abnormalities that might interfere with the study
(6) physical ability sufficient to allow performance of endurance exercise training
(7) capacity to provide written and dated informed consent form
(8) medical clearance to undergo a symptom-limited cardiopulmonary exercise test and extensive aerobic exercise training.

Participants recruited from the community via newspaper articles and community advertisement completed additional testing to determine MCI status. To distinguish between amnestic and non-amnestic MCI, agreed education adjusted cut-offs of -2 Standard Deviation (SD) for low education (<10 years of education), -1.5 SD for the middle group (10–13 years of education) and -1 SD for the highly educated (>13 years of education) will be taken from the delayed recall portion of an age adjusted episodic memory test. In Nijmegen and Dublin this will be evaluated using the Logical Memory (story recall) subtest of the Wechsler Memory Scale (WMS-IV)[REF]. In Cologne, cognitive scores adjusted for education were examined using the Repeatable Battery for the Assessment of neuropsychological Status (RBANS) Delayed Memory Index (Score of < 85).

**EXCLUSION**
Participants were deemed ineligible if they meet any of the following criteria:
(1) diagnosis of AD or other type of dementia
(2) history of familial early-onset dementia
(3) enrolment in any investigational drug study
(4) history in the past 2 years of epileptic seizures (participants with epilepsy who have been stable off medication or seizure free for 2 years may be included)
(5) any major psychiatric disorder (a clinical diagnosis of major depressive disorder, bipolar or schizophrenia)
(6) past history or MRI evidence of brain damage, including significant trauma, stroke, hydrocephalus, mental retardation, or serious neurological disorder
(7) carotid stent or severe stenosis
(8) history of myocardial infarction within previous year
(9) congestive heart failure (New York Heart Association Class II, III or IV)
(10) uncontrolled hypertension or hypotension (systolic blood pressure >200 mm Hg and/or diastolic blood pressure >110 mm Hg at rest)
(11) unstable cardiac, renal, lung, liver, or other severe chronic disease
(12) type 2 diabetes mellitus with hypoglycemia in the last 3 months
(13) significant history of alcoholism or drug abuse within last 10 years
(14) engagement in moderate-intensity aerobic exercise training for more than 30 min, 3 times per week, during past 2 years
(15) history of vitamin B12 deficiency or hypothyroidism (stable treatment for at least 3 months is allowed)

(16) serious or non-healing wound, ulcer, or bone fracture
In Cologne and Nijmegen, participants were invited to complete brain MRI scans. Participants with pacemakers or other medical metal devices were not eligible for MRI scanning as per standard procedures.

**Withdrawal of participants**
The investigator could decide to withdraw a subject from the study for urgent medical reasons. Subjects could leave the study at any time for any reason if they wish to do so, without any consequences. All primary analyses have been performed on an intention-to-treat basis with all randomized participants included in the primary analyses. Participants who withdraw from the study were invited to attend T1 and T2 assessments.

**Randomization, allocation, concealment and blinding**
Following baseline assessment, participants were randomized to one of three arms using a centrally controlled computer generated randomization list (for each country) generated by an independent statistician. Participants were randomized to one of three arms as per. At each centre, the investigators were blinded to allocation order and the treatment will be assigned using sealed envelopes based on order of recruitment. Outcome assessors and exercise trainers will not be blinded to the allocated treatment arm.

## Appendix B: Freesurfer guide

This guide will help you get through the process of downloading and installing FreeSurfer at first. Later on there will be explained what commands you can and should use (and want you cannot and shouldn't do). Hopefully this will help you figuring it all out.

First of all, you need to set up an account in DRE (digital research environment). You just type in 'dre' at the search machine of the umc portal and it will tell you what to do.
Fill in all the things you need and set the security level on **low**. This will make it easy for you to transfer files to your virtual environment from your desktop.
Whenever you get access to your DRE, on the umc portal you can find the files that are really helpful for setting up your machine. Follow those guides and you'll be able to set up the account. If not, options for help can be found in the guides.

When you can get in your virtual machine and it all is working fine, it's time to download FreeSurfer. It might be useful to let Ralph van Hoorn (manager of the DRE) know that you are going to download FreeSurfer, he might save you a lot of trouble by doing it for you. If you have to do it on your own then follow the following steps.
You do not have access to the internet on your virtual machine so here is what you have to do:

- Go to https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall
- Download the right version and safe it in a folder on you regular computer (like in downloads or on your G disk)
- When the download is completed, copy the downloaded file from the folder to your rsrch disk (for example in the folder documents).
- Go to your virtual machine
- You can find the file in the following folder: /mnt/workspace/documents).
- Start terminal
- Use cd /mnt/workspace/documents to go to the right folder
- Then use the following command: sudo tar xvf <name of the file>
- It will ask for your password, type this and press enter. It starts unpacking now
- When it is finished, ask for a license on the website and place that file in the FreeSurfer folder. You are now good to go!

With the installation of FreeSurfer you get some examples (in the FreeSurfer folder you'll find a subjects folder). On their website you can find you how to run those to see whether it all is working fine.
When it is, you are ready to start using FreeSurfer for you own data.

To begin with, some useful commands in FreeSurfer and what they do

| **Command** | **What it does** |
|---|---|
| cd /../.. | Opening a specific folder |
| cd .. | Going to the previous folder |
| ls | Shows you what is inside the folder you're in |
| gedit ~/.bashrc | Opens the bash file |
| source ~/.bashrc | Updates the bash file in terminal |

By using the gedit ~/.bashrc command, you can update your FREESURFER_HOME pathway and SUBJECTS_DIR. Make sure to always type in source ~/.bashrc to update terminal after you made changes in the bashrc file.
Your SUBJECTS_DIR should always be the pathway to where the files you're processing are in.

Before you can start analyzing you have to convert the MRI images from .IMA to .nii.gz. Make sure you are in the folder that the scans that you want to convert are in. You might want to select the images you need and place them in a different folder before you start. This way you don't have to search for them each time but you can easily select the first and last scan.
You can use cd /<pathway to the folder> for opening the right folder.

For example: cd /home/z931169/NeuroExercise/NL_XX_XM/NL_XX_XM_TX

Use the following command first

recon-all –s <subject name> -i <name of the first image of the MRI you need> -i <name of the last image of the MRI you need> -s <name of the folder you want it to be in>

For example:
recon-all –s NL_XX_XM_TX –i <name of the first image> -i <name of the last image> -s NL_XX_XM_TX_1

The images will be converted to a .mgz file. This file will be placed in the folder you set as your SUBJECT_DIR. When it processed the files without an error, you can open de folder you just made en go to the mri/orig folder. You should find two files, 001.mgz and 002.mgz. 002.mgz is the one you need for converting. Remember to open the folder first using cd.
You can use this command

mri_convert 002.mgz <name you want it to have>.nii.gz

For example
mri_convert 002.mgz NL_XX_XM_TX.nii.gz

If you look in the mri/orig folder now, you should find a .nii.gz file with the name you just gave it.
By using the command freeview, you can open the .nii.gz file and see if everything went well. The T1 and T2 image can be overlapped so you can see if it overlaps correctly. You also need to check if the scan quality is good enough. If not, you should find the image that influences the scan quality negatively and remove that from the images. You would thus have to start over with converting.

You will notice that some T1 scans have a grey background instead of black. You need to change it to black, otherwise the recon-all will give you an error. You can do this by opening the UNI_Images and INV2 images in freeview. Apply the INV2 as a mask to the UNI_Images and you will see that the background changes almost completely to black. If not, you can change the intensity threshold.
Save the file in the right folder and use that with converting.
Convert the images you want to use for the subject (e.g. the T1 and T2 images). Always keep in mind to be working in the folder where you placed the scans.

If everything converted without errors, you should be able to start the first analysis.
The command line is:

recon-all –s <new name for the folder of the processed subject> -i /<pathway to the first scan> -T2 /<pathway to the second scan> -all –cw256 –openmp <number of processor cores you want to use>

For example:
recon-all –s NL_XX_XM_RA –i /home/z931169/NeuroExercise/NL_XX_XM/NL_XX_XM_T1/NL_XX_XM_T1_1/mri/orig/NL_XX_XM_T1.nii.gz –T2 –i /home/z931169/NeuroExercise/NL_XX_XM/NL_XX_XM_T2/NL_XX_XM_T2_1/mri/orig/NL_XX_XM_T2.nii.gz –all –cw256 –openmp 4

The cw256 flag is to make sure that only images within a field of 256 by 256 are used. Anything above it will not be accepted by FreeSurfer. The openmp <number of processor cores you want to use> is to speed up the pipeline a little bit (with 2 cores it can take up to 24 hours per scan).

After the recon-all finishes, it should say it finished without any errors. If you go to the folder the processed subject is in and go to the stats folder you will find a file with the name aseg.stats. You can open this file in gedit and see what the calculated volumes are. If those volumes seem odd, check the processed data for the quality. Make sure the quality is good enough, otherwise you can edit the data and possibly re-run the subject.

However, it might say that it finished with errors. When this happens you should check out this website (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TroubleshootingData) that will tell you what to do for each error you could get. When you look at the error message in terminal, you will also find details of what went wrong and where to find the log file.

Sometimes when it is correcting for errors, a XL defect can be detected and this can take quite some time to overcome (e.g. 49 hours). You should then check the brainmask.mgz and see whether the skull stripping went well. If not, you can use one of the solutions to try to fix a bad skull strip. Although that doesn't always work and you are left with doing it yourself by hand in tkmedit.
How you can and should do this, is documented in the above mentioned link.

When the recon-all analysis went well, you can start with the second analysis. The one of the hippocampal subfields.
This analysis is done with the processed data and the T2 image. To command to use is:

recon-all –s <subjectname after first analysis> -hippocampal-subfields-T1T2 $SUBJECTS_DIR/<path to T2 image> T1T2

For example:
recon-all –s NL_XX_XM_RA –hippocampal-subfields-T1T2 $SUBJECTS_DIR/NL_XX_XM/NL_XX_XM_T2/NL_XX_XM_T2_1/mri/orig/NL_XX_XM_T2.nii.gz T1T2

It will probably take about one or two hours to run. The file with the volumes of the subfields is in the mri folder.
In my case the volumes that were calculated with the recon-all analysis were overestimated, but this was corrected with the hippocampal volumes analysis.

**Appendix C: Borg scale**

| Zwaarte belasting | Borgscore |
|---|---|
| | 6 |
| zeer zeer licht | 7 |
| | 8 |
| zeer licht | 9 |
| | 10 |
| tamelijk licht | 11 |
| | 12 |
| redelijk zwaar | 13 |
| | 14 |
| zwaar | 15 |
| | 16 |
| zeer zwaar | 17 |
| | 18 |
| zeer zeer zwaar | 19 |
| maximaal | 20 |

## Appendix D: Results

**Table 4** Descriptive statistics for the values of the volumes (in $mm^3$) and age (in years).

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | Std. Deviation | 2* Std. Deviation |
|---|---|---|---|---|---|---|
| Left hippocampus (T0) | 18 | 2393 | 3421 | 2852 | 305,6 | 611,2 |
| Left hippocampus (T1) | 18 | 2358 | 3574 | 2943 | 329,4 | 658,8 |
| Right hippocampus (T0) | 18 | 2373 | 3620 | 3045 | 380,8 | 761,6 |
| Right hippocampus (T1) | 18 | 2324 | 3781 | 3101 | 389,1 | 778,3 |
| Left dentate gyrus (T0) | 18 | 173 | 286 | 221 | 27,2 | 54,4 |
| Left dentate gyrus (T1) | 18 | 176 | 293 | 232 | 30,6 | 61,1 |
| Right dentate gyrus (T0) | 18 | 175 | 310 | 241 | 35,3 | 70,6 |
| Right dentate gyrus (T1) | 18 | 173 | 308 | 246 | 37,5 | 74,9 |
| Age | 18 | 57 | 85 | 71 | 8,4 | |
| Valid N (listwise) | 18 | | | | | |

**Table 5** Descriptive statistics for the change in volume (in $mm^3$) after 6 months.

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Δ Left hippocampus | 17 | -213 | 654 | 79,1 | 216,0 |
| Δ Right hippocampus | 17 | -756 | 721 | 55,1 | 356,1 |
| Δ Left dentate gyrus | 17 | -38 | 66 | 11,2 | 27,5 |
| Δ Right dentate gyrus | 17 | -78 | 65 | 4,9 | 37,0 |
| Valid N (listwise) | 17 | | | | |

**Table 6** Multiple regression analysis with the left hippocampal volume as dependent variable.

**Variables Entered/Removed[a]**

| Model | Variables Entered | Variables Removed | Method |
|---|---|---|---|
| 1 | Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes[b] | . | Enter |

a. Dependent Variable: Difference_LH_6M0M

b. All requested variables entered.

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,424[a] | ,180 | -,093 | 225,87832 |

a. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 134306,653 | 4 | 33576,663 | ,658 | ,633[b] |
| | Residual | 612252,204 | 12 | 51021,017 | | |
| | Total | 746558,858 | 16 | | | |

a. Dependent Variable: Difference_LH_6M0M

b. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 332,158 | 253,616 | | 1,310 | ,215 |
| | Exercise_group | 28,450 | 82,148 | ,103 | ,346 | ,735 |
| | Exercise_classes | -1,008 | 1,625 | -,183 | -,620 | ,547 |
| | Mean_CPM | -,882 | ,666 | -,383 | -1,325 | ,210 |
| | Difference_CPM_6M0M | -,713 | 1,216 | -,168 | -,586 | ,569 |

a. Dependent Variable: Difference_LH_6M0M

**Table 7** Multiple regression analysis with the right hippocampal volume as dependent variable.

**Variables Entered/Removed[a]**

| Model | Variables Entered | Variables Removed | Method |
|---|---|---|---|
| 1 | Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes[b] | . | Enter |

a. Dependent Variable: Difference_RH_6M0M

b. All requested variables entered.

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,338[a] | ,114 | -,181 | 387,06566 |

a. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 231295,317 | 4 | 57823,829 | ,386 | ,815[b] |
| | Residual | 1797837,886 | 12 | 149819,824 | | |
| | Total | 2029133,203 | 16 | | | |

a. Dependent Variable: Difference_RH_6M0M

b. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 555,990 | 434,598 | | 1,279 | ,225 |
| | Exercise_group | -132,267 | 140,769 | -,290 | -,940 | ,366 |
| | Exercise_classes | -1,357 | 2,785 | -,150 | -,487 | ,635 |
| | Mean_CPM | -,619 | 1,141 | -,163 | -,542 | ,597 |
| | Difference_CPM_6M0M | -,795 | 2,083 | -,114 | -,382 | ,709 |

a. Dependent Variable: Difference_RH_6M0M

**Table 8** Multiple regression analysis with the left dentate gyrus volume as dependent variable.

**Variables Entered/Removed[a]**

| Model | Variables Entered | Variables Removed | Method |
|---|---|---|---|
| 1 | Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes[b] | . | Enter |

a. Dependent Variable: Difference_LDG_6M0M

b. All requested variables entered.

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,568[a] | ,322 | ,097 | 26,12400 |

a. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 3897,165 | 4 | 974,291 | 1,428 | ,284[b] |
| | Residual | 8189,562 | 12 | 682,464 | | |
| | Total | 12086,727 | 16 | | | |

a. Dependent Variable: Difference_LDG_6M0M

b. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 62,309 | 29,332 | | 2,124 | ,055 |
| | Exercise_group | -2,840 | 9,501 | -,081 | -,299 | ,770 |
| | Exercise_classes | -,260 | ,188 | -,372 | -1,385 | ,191 |
| | Mean_CPM | -,117 | ,077 | -,401 | -1,525 | ,153 |
| | Difference_CPM_6M0M | ,072 | ,141 | ,133 | ,509 | ,620 |

a. Dependent Variable: Difference_LDG_6M0M

**Table 9** Multiple regression analysis with the right dentate gyrus volume as dependent variable.

**Variables Entered/Removed[a]**

| Model | Variables Entered | Variables Removed | Method |
|---|---|---|---|
| 1 | Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes[b] | . | Enter |

a. Dependent Variable: Difference_RDG_6M0M

b. All requested variables entered.

**Model Summary**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,527[a] | ,278 | ,037 | 36,34791 |

a. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 6103,749 | 4 | 1525,937 | 1,155 | ,378[b] |
| | Residual | 15854,048 | 12 | 1321,171 | | |
| | Total | 21957,798 | 16 | | | |

a. Dependent Variable: Difference_RDG_6M0M

b. Predictors: (Constant), Difference_CPM_6M0M, Exercise_group, Mean_CPM, Exercise_classes

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | 84,788 | 40,811 | | 2,078 | ,060 |
| | Exercise_group | -21,348 | 13,219 | -,450 | -1,615 | ,132 |
| | Exercise_classes | -,395 | ,262 | -,419 | -1,512 | ,157 |
| | Mean_CPM | -,069 | ,107 | -,175 | -,644 | ,532 |
| | Difference_CPM_6M0M | ,031 | ,196 | ,043 | ,159 | ,876 |

a. Dependent Variable: Difference_RDG_6M0M

Scans are left out of the analysis when they are below or above the following values (table 8) and are indicated with a red colour (table 9).

**Table 10** Cut-off points (in $mm^3$).

| | Min | Max |
|---|---|---|
| Left hippocampus (0M) | 2240,88932 | 3463,35648 |
| **Left hippocampus (6M)** | **2284,63690** | **3602,19230** |
| Right hippocampus (0M) | 2283,46984 | 3806,59836 |
| **Right hippocampus (6M)** | **2323,09824** | **3879,64836** |
| Left dentate gyrus (0M) | 166,56238 | 275,41802 |
| **Left dentate gyrus (6M)** | **171,33784** | **293,57196** |
| Right dentate gyrus (0M) | 170,78924 | 311,98836 |
| **Right dentate gyrus (6M)** | **171,36624** | **321,35336** |

**Table 11** Values of the structures (in $mm^3$) for each participant.

| | Left hippocampus HS | Right hippocampus HS | Left dentate gyrus | Right dentate gyrus |
|---|---|---|---|---|
| NL_02_0M | 2415,109828 | 2602,387198 | 193,722393 | 218,35001 |
| **NL_02_6M** | **2358,263924** | **2828,258846** | **197,032589** | **220,039039** |
| NL_03_0M | 3421,374486 | 3620,066246 | 285,72861 | 309,748287 |
| **NL_03_6M** | **3573,723796** | **3488,578546** | **293,425542** | **302,101187** |
| NL_05_0M | 2633,23486 | 2551,210886 | 173,280388 | 209,729588 |
| **NL_05_6M** | **2617,751879** | **2594,967745** | **215,671291** | **225,77398** |
| NL_06_0M | 3089,930856 | 3226,82477 | 217,565621 | 216,901258 |
| **NL_06_6M** | **3111,353167** | **3277,780195** | **210,181422** | **230,74439** |
| NL_08_0M | 2961,432284 | 3370,588135 | 232,139709 | 271,219117 |
| **NL_08_6M** | **2771,496006** | **2614,555777** | **209,106209** | **193,591907** |
| NL_10_0M | 2603,644084 | 2373,422119 | 189,125006 | 174,764727 |
| **NL_10_6M** | **2480,176056** | **2324,436834** | **175,562015** | **173,094681** |

| | | | | |
|---|---|---|---|---|
| NL_11_0M | 2464,877137 | 3330,816514 | 206,55831 | 254,677116 |
| **NL_11_6M** | **2809,382172** | **3780,870972** | **230,236666** | **279,507691** |
| NL_14_0M | 2908,898848 | 2629,66317 | 231,197389 | 220,139256 |
| **NL_14_6M** | **2696,02995** | **2553,2519** | **193,667958** | **202,648633** |
| NL_16_0M | 3369,124906 | 3241,559932 | 249,785844 | 247,776298 |
| **NL_16_6M** | **3256,460184** | **3280,740134** | **245,140012** | **260,643073** |
| NL_19_0M | 2980,114656 | 3071,459325 | 219,002619 | 234,155862 |
| **NL_19_6M** | **3123,974855** | **3287,743109** | **258,82621** | **275,242661** |
| NL_22_0M | 2810,257553 | 3399,206112 | 226,931201 | 262,082092 |
| **NL_22_6M** | **3464,022171** | **2903,851251** | **253,17924** | **226,512307** |
| NL_23_0M | 2990,54688 | 3064,284463 | 235,17546 | 232,488529 |
| **NL_23_6M** | **2963,575669** | **3081,77929** | **238,151237** | **238,266274** |
| NL_25_0M | 2443,784846 | 2574,607137 | 174,145431 | 178,982791 |
| **NL_25_6M** | **2714,365209** | **3295,380477** | **221,225974** | **244,103965** |
| NL_26_0M | 2875,509684 | 2981,364905 | 232,893359 | 281,052636 |
| **NL_26_6M** | **3028,546611** | **2994,478018** | **240,133395** | **265,097441** |
| NL_27_0M | 3002,757151 | 3238,042321 | 242,660107 | 267,40002 |
| **NL_27_6M** | **3065,793885** | **2968,15175** | **229,258654** | **212,303899** |
| NL_31_0M | 2393,382639 | 2682,357331 | 218,023956 | 229,127259 |
| **NL_31_6M** | **2576,114324** | **3233,18843** | **228,686484** | **272,498568** |
| NL_35_0M | 2985,025633 | 3430,871808 | 224,853112 | 274,823769 |
| **NL_35_6M** | **3051,644662** | **3457,782653** | **245,011699** | **291,376318** |
| NL_39_0M | 2989,206541 | 3421,881037 | 225,034245 | 261,57988 |
| **NL_39_6M** | **3173,006509** | **3650,404371** | **291,347543** | **307,740582** |

## Appendix E: Reflection personal goals

- *Analist/onderzoeker, carrière perspectief*

Oorzaak

De wetenschappelijke onderzoekswereld heeft mijn interesse vanwege de diversiteit en uitdaging van onderzoekswerkzaamheden. Ik wil hier mij heel graag op richten vanwege het goede toekomstperspectief. Tijdens deze afstudeeropdracht zal ik mij volledig in de rol van analist/onderzoeker bevinden.

Symptoom

Ik heb nog weinig ervaring met onderzoek, enkel met projecten tijdens de opleiding.

Leerdoel

Aan het einde van mijn afstuderen ben ik in staat om de verschillende stappen van het onderzoek te kunnen doorlopen en dit relatief zelfstandig te kunnen doen. De analyses die ik ga uitvoeren zijn ook noodzakelijk voor het onderzoek.

Acties

Verdiepen in de verschillende stappen van een onderzoek.
Leren oplossingsgericht te denken bij fouten (tijdens een meting).
Het leren uitvoeren van de analyses voor de MRI data.

**Reflectie**

Het is absoluut gelukt om mee oplossingsgericht te gaan denken en om heel zelfstandig te worden. Verschillende fouten heb ik zelf weten op te lossen, voornamelijk tijdens de analyses van de MRI scans.
Daarnaast ben ik ook veel meer bij het onderzoek betrokken geraakt, verschillende taken heb ik op mij genomen wat een duidelijk inzage gaf van wat er allemaal bij komt kijken en waar rekening mee gehouden moet worden.
Verder zijn alle analyses voor de MRI scans succesvol afgerond, waarbij ik dus wel kan zeggen dat het zeker is gelukt om te leren hoe ik deze uit moest voeren.

- *Communicatie*

Oorzaak

Ik heb nu nog moeite met het goed op papier kunnen zetten van wat ik wil zeggen.
Mondeling gaat dit stukken beter dan in een verslag.

Symptoom

Wat ik eigenlijk bedoel komt verkeerd of anders over, wat tot misverstanden leidt.

Leerdoel

Het goed leren omschrijven van waarom iets gebeurd, hoe en leren om dit goed te kunnen onderbouwen met duidelijk voorbeelden en bronnen.

Acties

Tijdens de (tussentijdse) terugkoppeling met de afstudeerbegeleider dit schriftelijk goed te kunnen bewoorden.
Tips van docenten, afstudeerbegeleiders en collega's vragen.
Tijdens onderzoeksbesprekingen op de afstudeerplek de verschillende onderdelen van het project presenteren en de feedback hierop meenemen.

**Reflectie**

Voor veel problemen tijdens het project heb ik via de mail contact op moeten nemen, wat er zeker voor heeft gezorgd dat mijn schriftelijke communicatie (kort maar duidelijk kunnen verwoorden) vooruit is gegaan. Op de afdeling werd er ook wekelijks een opening gehouden waarin vragen/opmerkingen/problemen werden besproken, wat ook heeft bijgedragen aan het beter leren van het vertellen/uitleggen van situaties.
Verder heb ik eens in de twee weken overleg gehad met mijn begeleider wat er voor zorgde dat ik veel heb kunnen overleggen en vertellen, wat voor mij heel prettig heeft gewerkt.

- *ICT*

Oorzaak

Tijdens de afgelopen jaren op de opleiding had ik wat moeite met programma's als SPSS en Matlab.

Symptoom

Tijdens mijn eerste stage heb ik niet met die programma's gewerkt, tijdens mijn tweede stage heb ik met SPSS gewerkt.

Leerdoel

Ik wil graag meer kennis opdoen van verschillende programma's. Tijdens dit afstudeerproject zal ik met name SPSS en een ander programma (Freesurfer) gebruiken. Hoewel in Freesurfer ook verschillende commands worden gebruikt, is dit toch anders en wil ik het graag leren.

Acties

De beschreven handleiding zelf doornemen
Uitleg krijgen van verschillende personen die ervaring hebben met Freesurfer om de analyses volledig zelfstandig uit te kunnen voeren

**Reflectie**

Het heeft veel moeite gekost om het programma goed te begrijpen en kunnen gebruiken. Hiervoor heb ik hulp gehad van het Donders Instituut, Michel Hu, Jan-Willem Thielen en Daan de Jong. Allemaal hebben zij mij met verschillende onderdelen mij kunnen helpen.
Daarnaast heb ik zelf een nieuwe handleiding geschreven. Deze is iets specifieker dan de vorige en geeft een inzicht van de commands die worden gebruikt in FreeSurfer.

**Appendix F: Research proposal**

# The influence of exercising on hippocampal volume in elderly with Mild Cognitive Impairment (MCI)

## A pilot study


| | |
|---|---|
| Naam | Ida van de Water |
| Studentnummer | 14013649 |
| Email | 14013649@student.hhs.nl |
| Datum | 15 januari 2018 |
| Werkveld | Gezondheidszorg/onderzoek |
| Beroepsrol | Onderzoeksassistent |
| Opdrachtgever | Radboudumc, afdeling geriatrie |
| Eerste begeleider | Antoon Dobbelsteen |
| Periode | 19-2-2018/15-6-2018 |

| Studievoortgang | |
|---|---|
| ***Minor*** | Translational Neuroscience (Radboud Universiteit Nijmegen) |
| ***Stage II*** | Temporal order memory in patients with MCI (Radboudumc) |
| ***Bepaalde studiepunten module 9 t/m 11*** | 33 |
| ***Bepaalde vrije studiepunten*** | 15 |
| ***Openstaande toetsen*** | 0 |

**De invloed van sporten op neurogenese in de hippocampus.**

*Werkveld:* Onderzoek in de gezondheidzorg
Onderzoeksassistent
*Extern project:* Ja
*Bedrijf:* Radboudumc, afdeling geriatrie
*Contactpersoon:* Marit Sanders, marit.sanders@radboudumc.nl

# Inleiding

Alzheimer is de meest voorkomende neurodegeneratieve aandoening, wat wil zeggen dat de ziekte progressief is en er steeds meer zenuwcellen zullen afsterven. Gezien de wereldpopulatie steeds ouder wordt, zal de prevalentie steeds meer stijgen (He, Goodkind, & Kowal, 2016). Alzheimer is één van de vormen van dementie, en in dit geval de meest voorkomende vorm. Het verloop van gezond verouderen naar een vorm van dementie is niet heel plotseling. De eerste veranderingen kunnen vele jaren voor het uiten van de ziekte al aanwezig zijn. Deze eerste veranderingen kunnen aangeduid worden met Mild Cognitive Impairment (MCI). De diagnose MCI houdt in dat patiënten geheugen klachten hebben die niet onder het gezond verouderen vallen, maar ze zijn nog niet beperkt in het dagelijks functioneren. MCI wordt gezien als een fase, die tussen het normaal cognitief verouderen (lichte afname van sommige cognitieve functies maar het behouden van de volledige zelfstandigheid) en het dementeren valt. De diagnose dementie kan worden gesteld, volgens de DSM V handleiding, als er in meerdere cognitieve domeinen een ernstige achteruitgang plaatsvindt en wanneer algemene dagelijkse handelingen niet meer zelfstandig kunnen worden uitgevoerd. Daarnaast mag het ook niet te wijten zijn aan een delirium of aan een andere psychische stoornis.

Mensen met de diagnose MCI kunnen verslechteren op het gebied van cognitie en het zelfstandig kunnen uitvoeren van algemene dagelijkse handelingen, waardoor ze dementie kunnen ontwikkelen. Ze kunnen echter ook stabiel blijven of zelfs verbeteren wat hun algemene cognitie betreft (Smith & Bondi, 2013, p. 73). Mensen met MCI hebben nog geen ernstige beperkingen in het dagelijks functioneren waardoor het een geschikte groep kan zijn om te zien of het verloop naar dementie vertraagd kan worden.

Een mogelijke manier om dementie te vertragen, kan zijn door lichaamsbeweging (Ahlskog et al., 2011). Verondersteld wordt dat ouderen voldoende bewegen wanneer zij minimaal 5 dagen per week 30 minuten met een gemiddelde intensiteit sporten (Garber et al., 2011). Uit een review studie van onderzoeken naar het effect van sporten op cognitie bij ouderen met MCI, blijkt dat veel studies heel verschillend zijn in opzet en uitkomst. Hierdoor kan er nog geen duidelijk beeld geschetst worden wat sporten voor invloed heeft op de cognitie (Öhman, Savikko, Strandberg, & Pitkälä, 2014). Het wordt echter wel aangeraden om sporten als één van de behandelmogelijkheden tegen MCI te gebruiken (Langa & Levine, 2014; Petersen et al., 2018).
Om hier duidelijkheid in te scheppen is er een studie opgezet, ‘’The effects of an extensive exercise program on the progression of Mild Cognitive Impairment (MCI)’’, waar naar gerefereerd zal worden als het Bewegend Brein. Deze studie vindt plaats op drie verschillende locaties, waarvan het

Alzheimer centrum van het Radboudumc in Nijmegen er een van is. Het is een longitudinale studie die opgezet is als een gerandomiseerd gecontroleerd experiment.

Het Bewegend Brein is een studie die na een trainingsprogramma van 12 maanden gaat kijken wat dit doet met algemene cognitie, cardiovasculaire fitheid en fysieke fitheid. De algemene cognitie wordt gemeten met de Montreal Cognitive Assessment (MoCA). De fysieke fitheid wordt gemeten met een fietstest (Devenney et al., 2017).
Naast de algemene cognitie is er een extra test ontworpen voor de deelnemers, die zich focust op het temporele geheugen. Het temporele geheugen wordt gebruikt om activiteiten in het verloop van de tijd in de juiste volgorde op te slaan. Een van de andere testen is een MRI scan van het brein. Deze scan wordt op twee verschillende momenten gemaakt, voordat de deelnemers starten met het programma en na 6 maanden in het programma. Door het maken van een MRI scan kan er gekeken worden naar anatomische locaties in het brein, waaronder de hippocampus. De hippocampus hangt sterk samen met het geheugen en het is daarom interessant om hiernaar te kijken.

De resultaten van deze MRI scans kunnen gebruikt worden om te zien wat de invloed van sporten is op de hippocampus. Er zijn studies die hebben aangetoond dat het volume van de hippocampus in patiënten met Alzheimer is afgenomen in vergelijking met een controle groep zonder cognitieve stoornissen. Onderzoek met patiënten met MCI hebben ook een afname van het volume van de hippocampus laten zien vergeleken met de controle groep, maar een minder grote afname dan de Alzheimer patiënten (Shi, Liu, Zhou, Yu, & Jiang, 2009). Een afname in het volume van de hippocampus kan dus een aanwijzing zijn dat MCI een voorstadium is van dementie (Driscoll et al., 2009; Convit et al., 1997). Het blijft echter moeilijk om te voorspellen wie er daadwerkelijk cognitief zal verslechteren. Hoeveel tijd er overheen gaat om tot het dementie stadium te verslechteren is ook onduidelijk.

Een andere manier om cognitief functioneren te bekijken is met behulp van neuropsychologische testen. De verschillende vormen van dementie beginnen op verschillende plekken in het brein, waarvan de meest voorkomende vorm van dementie (Alzheimer), begint in de hippocampus. Een vorm van geheugen die gerelateerd is aan de hippocampus is het temporele geheugen. Het temporele geheugen zou ook als eerste afnemen wanneer personen de MCI fase bereiken (Devito & Eichenbaum, 2011; Fortin et al., 2002; Galasko & Gilbert, 2013; Hsieh et al., 2014).
Ook het temporele geheugen kan getest worden met een neuropsychologische test. Deze test is voor de Bewegend Brein studie ontworpen, zodat getest kan worden in een MCI populatie of een beweegprogramma leidt tot een verbeterde score. De invloed van het sporten op het temporele geheugen is onderzocht tijdens de tweede stage met de ontworpen neuropsychologische test. Om de invloed van het sporten (zowel aeroob als anaeroob) op het temporele geheugen te testen, is er gekeken naar de relatie tussen de twee sportgroepen en de score van de temporele geheugen test. De hypothese was dat de score in de sport groepen hoger zou zijn dan in de controle groep, doordat het sporten een volumetoename van de hippocampus zou initiëren en het temporele geheugen aan het hippocampusvolume is gekoppeld. De resultaten lieten bij zowel aeroob als anaeroob sporten geen verbetering in het temporele geheugen zien in vergelijking met de controle groep.

De hypothese was gebaseerd op de relatie tussen het temporele geheugen en het hippocampus volume, dus nu is het onduidelijk of de test niet gevoelig genoeg is om het temporele geheugen te kunnen vastleggen, of dat er geen toename van het hippocampus volume is door het sporten.

In verouderde muizen is er aangetoond dat er door te sporten neurogenese, de ontwikkeling en groei van nieuwe neuronen, extra wordt gestimuleerd in de hippocampus, wat leidt tot een toename van het hippocampus volume (Brown et al., 2003; van Praag, 2005). Of sporten ook bij mensen tot een volume toename van de hippocampus leidt, is nog onduidelijk. Er zijn resultaten beschreven van ouderen met geheugenklachten waarbij degenen die meer stappen (>4000) zetten op een dag een

groter hippocampus volume (in $mm^3$) hadden (Siddarth et al., 2017). Bewegen lijkt dus een invloed te hebben op het hippocampus volume. Om dit te onderzoeken kan het volume van de hippocampus worden bepaald voor en na een periode van bewegen. Verschillende studies wijzen erop dat sporten de afname van het hippocampus volume remt, en er zo voor zorgt dat ouderen een grotere cognitieve reserve hebben. Dit betekent dat het verloop van MCI naar dementie uitgesteld zou kunnen worden doordat het volume van de hippocampus niet verder afneemt (K. I. Erickson et al., 2011; Firth et al., 2018).

Om het volume van de hippocampus te kunnen bepalen in een MCI groep die veel aan bewegen doet, wordt de data van het Bewegend Brein gebruikt. Het Bewegend Brein kent 42 deelnemers en zij werden gerandomiseerd in een van de drie groepen geplaatst. Dit zijn de aerobe groep, de anaerobe groep en de controle groep.
Voordat de deelnemers beginnen aan het programma, wordt er een MRI scan van het brein gemaakt. Deze scan wordt herhaald na 6 maanden sporten. In deze afstudeeropdracht zullen de volumes van de hippocampus van beide momenten worden bepaald om antwoord te kunnen geven op de volgende onderzoeksvraag:

*Wat is de invloed van 6 maanden aeroob en anaeroob sporten op het volume van de hippocampus ($mm^3$) in vergelijking met de controle groep?*

De deelvragen zijn:
*Leidt een toename van het hippocampus volume tot een hogere score op de temporele geheugen taak?*
*Leidt een toename van het hippocampus volume tot een hogere score van de algemene cognitie, gemeten met de MoCA?*

Dat door bewegen het hippocampus volume kan toenemen wordt in verschillende studies gevonden (Kirk I Erickson et al., 2011; Firth et al., 2018; Siddarth et al., 2017) wat leidt tot de hypothese dat ongeacht de vorm van bewegen (aeroob of anaeroob) er een vergroting van het volume van de hippocampus door neurogenese plaatsvindt.

# Methode

Totaal zijn er 42 deelnemers die aan de studie deelnemen. Het is een longitudinale studie waardoor iedere deelnemer is op een ander tijdstip gestart. Enkelen zijn al volledig klaar met het programma en de laatste deelnemers zijn in maart/april 2018 op de helft (6 maanden in het programma). In bijlage A kan een tijdsverloop van de Bewegend Brein studie worden gevonden.
De MRI data van alle deelnemers van zowel de meting voorafgaand aan het sporten (T0) en de 6 maanden (T1) meting zal dus beschikbaar zijn vanaf april 2018. Tijdens dit afstudeerproject zal ik de MRI data gaan analyseren in het programma Freesurfer. Met dit programma kan er een schatting worden gemaakt van het volume van de hippocampus in $mm^3$. Het verschil in volume zal met SPSS statistisch getoetst worden.

## Deelnemers

De inclusie voor de studie is geëindigd in oktober 2017. Potentiële kandidaten zijn na een vrijwillige aanmelding gebeld om te zien of zij mogelijk geschikt zouden zijn voor de studie. Kandidaten die aan de telefonische screening voldeden, werden uitgenodigd in het Radboudumc voor een uitgebreidere screening.
Hier werd onder andere de MoCA test afgenomen waarvan de score tussen de 18 en 26 moest liggen voor deelname. Een score lager dan 26 uit 30 duidt op geheugenklachten die niet meer onder het gezond verouderen vallen. Een score lager dan 18 kan op een mogelijke dementie duiden.
Kandidaten mochten de afgelopen 2 jaar ook niet gestructureerd gesport hebben maar moesten wel in staat zijn om te sporten. Voor de volledige in- en exclusie criteria, zie bijlage B.

## Programma

Deelnemers die in de studie zijn geïncludeerd, kregen na het eerste onderzoek te horen in welke groep ze ingedeeld waren (aeroob, anaeroob of controle). Deze indeling is gerandomiseerd. Voor de sport groepen (aeroob en anaeroob) betekende dit dat zij ingedeeld werden bij bestaande beweeggroepen. Deze beweeggroepen waren allemaal onderdeel van reeds bestaande senioren sport groepen met docenten met een speciale senioren opleiding. Enkele sporten die worden beoefend door de anaerobe groep zijn qi gong en yoga. Voorbeelden van aerobe groepen zijn, sport en spel en bootcamp. De deelnemers in de sportgroepen sporten 3 keer per week, 12 maanden lang.

Om te controleren hoe vaak de personen daadwerkelijk sporten en op welke intensiteit ze dit doen, wordt elke deelnemer maandelijks bezocht bij een beweegles. Tijdens deze lessen wordt de hartslag opgenomen en een BORG score gevraagd. De BORG score is een subjectieve schaal, waarop de deelnemer kan aanduiden hoe zwaar het sporten voor hen is. De BORG schaal is bijgevoegd in bijlage C. Voor de aerobe en anaerobe groep zijn streefwaardes opgesteld voor de hartslag en BORG score. Door dit tijdens een les op te nemen kan er dus gekeken worden of de deelnemers in de buurt komen van de opgestelde streefwaardes.
Verder houden deelnemers een beweegdagboek bij. Hierin vermelden zij elke sportieve activiteit die ze hebben gedaan. De deelnemers noteren wat voor sport ze hebben gedaan, de duur van de sport en hoe zwaar ze de activiteit vonden, middels de BORG score. Elke keer wanneer een deelnemer wordt gezien, wordt er gevraagd of het beweegdagboek wordt ingevuld.

Ook worden er op 3 verschillende moment een om de pols gedragen beweegmonitor (Actiwatch) meegegeven. Deze meet een week lang de bewegingen van de deelnemer uitgedrukt in counts per minuut. De deelnemers dragen een beweegmonitor op T0 en T1. Uit deze data kan de intensiteit, duur en frequentie van de beweegmomenten berekend worden. Hiermee kan ook het beweegpatroon van de controle groep bepaald worden. Deze uitkomsten zullen meegenomen worden in de statistische analyses.

## MRI scan

De MRI scan wordt gemaakt in het Donders Instituut in Nijmegen, met een 3-T MRI scanner (Siemens Magnetom Prisma syngo MR D13D). Deze scanner maakt gedetailleerde beelden van anatomische details van het brein.

## Data-analyse

Met het programma Freesurfer 6.0 worden de MRI scans geanalyseerd. Dit programma maakt een semi-automatische subcorticale segmentatie van het brein. Dit wil zeggen dat er volgens een standaard functie in Freesurfer het brein wordt opgedeeld in de algemene bekende structuren, waaronder de hippocampus. Met dit programma kan dus de hippocampus in kaart worden gebracht en kan er een schatting van het volume in $mm^3$ gemaakt worden.

Voordat de analyse in Freesurfer gestart kan worden, moeten de beelden van de scans voorbereid worden. De eigenschappen van de scan moeten aangepast worden om de gewenste analyse mogelijk te maken. Voor het programma Freesurfer is er een basis handleiding geschreven voor de installatie en het gebruik van Freesurfer, zie bijlage D. Na de installatie kunnen de scans via verschillende commands in het programma Freesurfer worden geladen. Na het handmatig controleren van de beeldkwaliteit kan de algemene analyse worden gestart. Afhankelijk van de computer duurt dit 7 tot 24 uur per scan analyse.
Wanneer deze analyse klaar is, moet er handmatig worden gekeken naar de stappen die doorlopen zijn. Er wordt handmatig gekeken naar de geïdentificeerde structuren van de hippocampus, de stappen die het programma tijdens de analyse heeft gemaakt worden volledig doorlopen en de uitkomst wordt gecontroleerd.
Dit is erg belangrijk omdat het programma 'harde' fouten kent, dan is de analyse niet volledig uitgevoerd. En het programma kent 'zachte' fouten, dan is de scan uitgevoerd naar kunnen er alsnog fouten in zitten. Deze fouten moeten dan eerst opgelost worden voordat de resultaten gebruikt kunnen worden. Dit staat ook beschreven in de basishandleiding (bijlage D).

De gevonden volumewaardes zullen in SPSS worden ingevoerd van de T0 en T1 MRI. In SPSS zal worden gekeken of de data normaal verdeeld is en er moet gekeken worden naar de missende data. Hierna zullen verschillende statistische testen volgen. Waarschijnlijk zal er een ANOVA test worden gedaan om te bepalen of de groepen (aeroob, anaeroob en controle) een verschil hebben bij de baseline. Er zal ook een ANOVA worden gebruikt om te bepalen of het beweegniveau, berekend met BORG score, hartslag en data van de Actiwatch, gerelateerd is aan het hippocampus volume.
Een multiple regressie model zal worden gemaakt om te bepalen of het sporten leidt tot een significant verschil in volume tussen de groepen. De afhankelijke variabele is in dit geval het verschil van het volume van de hippocampus na 6 maanden sporten en de onafhankelijke variables zijn de groepen en covariaties (leeftijd, geslacht, beweegniveau). Er wordt een significant verschil gevonden in de geteste populatie wanneer $p < 0{,}05$.

## Neven activiteiten

Naast de afstudeeropdracht, het bepalen van het volume van de hippocampus van de deelnemers voordat ze begonnen met sporten en na dat ze 6 maanden aan het sporten waren, zal ik onderdeel zijn van een multidisciplinair team.
In dit team zitten geriaters, neuropsychologen, een technisch geneeskundige, een sport en bewegen student en geneeskunde studenten. In bijlage A, waar het tijdsverloop van de Bewegend Brein studie is weergeven, is te lezen dat de laatste deelnemers in oktober 2018 het jaar van deelname aan de studie hebben doorlopen.
Binnen dit multidisciplinaire team zal ik dan ook assisteren bij metingen, contact onderhouden met deelnemers wanneer nodig en hen bezoeken bij beweeglessen.

## Beroepsproduct

Het product dat uit dit afstudeerproject zal volgen, zal een eerste versie van een Engelstalig artikel zijn. Dit zal een artikel zijn waarin wordt beschreven of sporten tot een vergroting van het volume van de hippocampus kan leiden. Als dit wordt gevonden, kan er ook worden beschreven welk beweegniveau aangehouden moet worden om het volume van de hippocampus te kunnen vergroten. Daarnaast zal er ook een uitvoerige beschrijving worden gemaakt van het gebruik van het programma Freesurfer en hoe het volume van de hippocampus bepaald is.

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# Persoonlijke leerdoelen

- *Analist/onderzoeker, carrière perspectief*

Oorzaak

De wetenschappelijke onderzoekswereld heeft mijn interesse vanwege de diversiteit en uitdaging van onderzoekswerkzaamheden. Ik wil hier mij heel graag op richten vanwege het goede toekomstperspectief. Tijdens deze afstudeeropdracht zal ik mij volledig in de rol van analist/onderzoeker bevinden.

Symptoom

Ik heb nog weinig ervaring met onderzoek, enkel met projecten tijdens de opleiding.

Leerdoel

Aan het einde van mijn afstuderen ben ik in staat om de verschillende stappen van het onderzoek te kunnen doorlopen en dit relatief zelfstandig te kunnen doen. De analyses die ik ga uitvoeren zijn ook noodzakelijk voor het onderzoek.

Acties

Verdiepen in de verschillende stappen van een onderzoek.
Leren oplossingsgericht te denken bij fouten (tijdens een meting).
Het leren uitvoeren van de analyses voor de MRI data.

- *Communicatie*

Oorzaak

Ik heb nu nog moeite met het goed op papier kunnen zetten van wat ik wil zeggen.
Mondeling gaat dit stukken beter dan in een verslag.

Symptoom

Wat ik eigenlijk bedoel komt verkeerd of anders over, wat tot misverstanden leidt.

Leerdoel

Het goed leren omschrijven van waarom iets gebeurd, hoe en leren om dit goed te kunnen onderbouwen met duidelijk voorbeelden en bronnen.

Acties

Tijdens de (tussentijdse) terugkoppeling met de afstudeerbegeleider dit schriftelijk goed te kunnen bewoorden.
Tips van docenten, afstudeerbegeleiders en collega's vragen.
Tijdens onderzoeksbesprekingen op de afstudeerplek de verschillende onderdelen van het project presenteren en de feedback hierop meenemen.

- *ICT*

Oorzaak

Tijdens de afgelopen jaren op de opleiding had ik wat moeite met programma's als SPSS en Matlab.

Symptoom

Tijdens mijn eerste stage heb ik niet met die programma's gewerkt, tijdens mijn tweede stage heb ik met SPSS gewerkt.

Leerdoel

Ik wil graag meer kennis opdoen van verschillende programma's. Tijdens dit afstudeerproject zal ik met name SPSS en een ander programma (Freesurfer) gebruiken. Hoewel in Freesurfer ook verschillende commands worden gebruikt, is dit toch anders en wil ik het graag leren.

Acties

De beschreven handleiding zelf doornemen

Uitleg krijgen van verschillende personen die ervaring hebben met Freesurfer om de analyses volledig zelfstandig uit te kunnen voeren.

# Planning

| Weken (2018) | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Literatuurstudie | | | | | | | | | | | | | | | | | | | | | |
| *Literatuur algemeen* | | | | | | | | | | | | | | | | | | | | | |
| *Literatuur freesurfer* | | | | | | | | | | | | | | | | | | | | | |
| Methode | | | | | | | | | | | | | | | | | | | | | |
| *Methode freesurfer beschrijven* | | | | | | | | | | | | | | | | | | | | | |
| *Methode SPSS beschrijven* | | | | | | | | | | | | | | | | | | | | | |
| *Methode MRI data beschrijven* | | | | | | | | | | | | | | | | | | | | | |
| Resultaten | | | | | | | | | | | | | | | | | | | | | |
| *Analyseren data freesurfer* | | | | | | | | | | | | | | | | | | | | | |
| *Analyseren data SPSS* | | | | | | | | | | | | | | | | | | | | | |
| *Reflectie data* | | | | | | | | | | | | | | | | | | | | | |
| Verslagtechnisch | | | | | | | | | | | | | | | | | | | | | |
| Discussie & Conclusie | | | | | | | | | | | | | | | | | | | | | |
| Schrijfdagen | | | | | | | | | | | | | | | | | | | | | |

**Het afstudeerwerk moet in week 24 zijn ingeleverd (eindversie).**

**Het streven is om in week 22 de eerste versie van het verslag in te leveren voor feedback.**
**In week 26 vinden de eindgesprekken plaats.**

# Bijlagen

## Bijlage A: Tijdsverloop studie

**Start eerste deelnemers:** mei 2016 **Einde eerste deelnemers:** mei 2017
**Start laatste deelnemers:** oktober 2017 **Einde laatste deelnemers:** oktober 2018

| **Algemene opzet studie** | | |
|---|---|---|
| T0 (baseline, 0 maanden) | T1 (6 maanden) | T2 (12 maanden → einde programma) |
| *Uitgebreide screening* | *Herhaling MRI scan en hersendoorbloeding* | *Herhaling metingen aan het vaatstelsel en hersendoorbloeding* |
| *Baseline metingen (MRI, metingen aan het vaatstelsel en hersendoorbloeding)* | | *Evaluatiegesprekken met deelnemers* |
| *Indeling groepen (aeroob, anaeroob of controle)* | | |

Een algemene schatting van het tijdsverloop van de volledige studie. Het begin (0 maanden) is januari 2015.

| Tijdsverloop (in maanden) | 0 | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|
| Voorbereidingen | | | | | | | | | |
| Inclusie | | | | | | | | | |
| Programma | | | | | | | | | |
| Verwerking resultaten | | | | | | | | | |

# Bijlage B: In- en exclusiecriteria

**INCLUSION**
Participants who meet the following criteria will be eligible to participate:
(1) MoCA 18–26
(2) stable medical condition for more than 6 months
(3) stable medication for more than 3 months
(4) adequate visual and auditory acuity to complete neuropsychological testing
(5) electrocardiogram without significant abnormalities that might interfere with the study
(6) physical ability sufficient to allow performance of endurance exercise training
(7) capacity to provide written and dated informed consent form
(8) medical clearance to undergo a symptom-limited cardiopulmonary exercise test and extensive aerobic exercise training.

Participants recruited from the community via newspaper articles and community advertisement will complete additional testing to determine MCI status. To distinguish between amnestic and non-amnestic MCI, agreed education adjusted cut-offs of -2 Standard Deviation (SD) for low education (<10 years of education), -1.5 SD for the middle group (10–13 years of education) and -1 SD for the highly educated (>13 years of education) will be taken from the delayed recall portion of an age adjusted episodic memory test. In Nijmegen and Dublin this will be evaluated using the Logical Memory (story recall) subtest of the Wechsler Memory Scale (WMS-IV). In Cologne, education scores will be examined using the Repeatable Battery for the Assessment of neuropsychological Status (RBANS) Delayed Memory Index (Score of < 85).

**EXCLUSION**
Participants will be deemed ineligible if they meet any of the following criteria:
(1) diagnosis of AD or other type of dementia
(2) history of familial early-onset dementia
(3) enrollment in any investigational drug study
(4) history in the past 2 years of epileptic seizures (participants with epilepsy who have been stable off medication or seizure free for 2 years may be included)
(5) any major psychiatric disorder (a clinical diagnosis of major depressive disorder, bipolar or schizophrenia)
(6) past history or MRI evidence of brain damage, including significant trauma, stroke, hydrocephalus, mental retardation, or serious neurological disorder
(7) carotid stent or severe stenosis
(8) history of myocardial infarction within previous year
(9) congestive heart failure (New York Heart Association Class II, III or IV)
(10) uncontrolled hypertension or hypotension (systolic blood pressure >200 mm Hg and/or diastolic blood pressure >110 mm Hg at rest)
(11) unstable cardiac, renal, lung, liver, or other severe chronic disease
(12) type 2 diabetes mellitus with hypoglycemia in the last 3 months
(13) significant history of alcoholism or drug abuse within last 10 years
(14) engagement in moderate-intensity aerobic exercise training for more than 30 min, 3 times per week, during past 2 years
(15) history of vitamin B12 deficiency or hypothyroidism (stable treatment for at least 3 months is allowed)
(16) serious or non-healing wound, ulcer, or bone fracture

In Cologne and Nijmegen, participants will be invited to complete brain MRI scans. Participants with pacemakers or other medical metal devices will not be eligible for MRI scanning as per standard procedures.

**Withdrawal of participants**

The investigator can decide to withdraw a subject from the study for urgent medical reasons. Subjects can leave the study at any time for any reason if they wish to do so without any consequences. All primary analyses will be performed on an intention-to-treat basis with all randomized participants included in the primary analyses. Participants who withdraw from the study will be invited to attend T1 and T2 assessments. Randomization, allocation, concealment and blinding Following baseline assessment, participants will be randomized to one of three arms using a centrally controlled computer generated randomization list (for each country) generated by an independent statistician. Participants will be randomized to one of three arms as per. At each centre, the investigators will be blinded to allocation order and the treatment will be assigned using sealed envelopes based on order of recruitment. Outcome assessors and exercise trainers will not be blinded to the allocated treatment arm.

Bijlage C: BORG schaal

| Zwaarte belasting | Borgscore |
|---|---|
| | 6 |
| zeer zeer licht | 7 |
| | 8 |
| zeer licht | 9 |
| | 10 |
| tamelijk licht | 11 |
| | 12 |
| redelijk zwaar | 13 |
| | 14 |
| zwaar | 15 |
| | 16 |
| zeer zwaar | 17 |
| | 18 |
| zeer zeer zwaar | 19 |
| maximaal | 20 |

# Bijlage D: Beschrijving Freesurfer

**Beginner's Guide for Freesurfer**
**by Michel Hu**

This guide will only focus on installing and setting up Freesurfer 6.0 on a Windows based system. In the case of Linux and Mac based systems you can just visit the website of Freesurfer and they will have a tutorial on how to install and set it up over there.

The first step in the process of installing Freesurfer 6.0 is to first set up a virtual machine on your computer. This virtual machine will allow you to install a different operating system on your computer.
The reason this is necessary is because Freesurfer 6.0 will only work on Linux based systems and Max and not windows. If you are thinking about using the Linux Bash Shell that is available with the windows 10 update it will not work with Freesurfer as it misses a lot of required libraries for Freesurfer to work. Safe yourself the trouble and install a virtual machine. I will preface this by saying that if you do start to use the virtual machine that the computer that you're working on needs to have high RAM and a lot of hard disk space. The virtual machine that you are creating will use the same resources as the host machine has and they for the most part work independently from each other. Therefore before even starting to set up Freesurfer make sure you have the requirements to run Freesurfer 6.0. The requirements for Freesurfer can be found here: https://surfer.nmr.mgh.harvard.edu/fswiki/SystemRequirements.
The most important aspect is to have more than 8GB of RAM memory on your host machine so you can allocate at least 8GB of memory to the virtual machine while maintaining some for the host machine so everything doesn't go extremely slow.

**Setting up the virtual machine**
After establishing that you have met the requirements for Freesurfer 6.0 it is time to download VirtualBox. This program will allow you to set up the virtual machine. Go to https://www.virtualbox.org/wiki/Downloads and download the most recent package for windows hosts. Also go to http://nl.releases.ubuntu.com/16.04/ and download the desktop image for Ubuntu 16.04. In most cases you will download the i386 image. This image disk is required for later on. After installation, open VirtualBox and you will be met with the home screen of VirtualBox. Click on new and give your virtual machine a name and choose Linux and select Ubuntu. The next step is to allocate the RAM memory. As I mentioned before you need at least 8GB, the more the better. After which you can select to create a virtual hard disk now. The Hard disk file type should be VDI (VirtualBox Disk Image). The next step will ask you what kind of hard disk you want it to be. The reason why I mention at the start to have a lot of hard disk space is for this. In my experience the dynamically allocated option doesn't work and I always chose the fixed size option. The problem is that you cannot alter the size after creation. So if you discover later on in your research that you lack in space than you need to create another virtual machine. So of importance is to establish beforehand how much disk space you will need and add some extra space to it. The biggest advantage of this option is that it will not take additional resources as it doesn't have to communicate between your host machine and virtual machine continuously. The last step to allocate

the amount of hard disk space you need and press create to create your own virtual machine. You will have a computer within a computer (Computerception)!

**Installing Linux in your virtual machine**

After the creation of the virtual machine is finished start it. The first prompt will ask you to select a start-up disk and here is where the Ubuntu 16.04 image disk comes into play. Select the Ubuntu 16.04 image disk and after all the loading is complete you will be greeted with an installation screen of Ubuntu 16.04. Install Ubuntu 16.04 and select the Download updates while installing Ubuntu option followed by the Erase disk and install Ubuntu option. Don't worry this will not erase windows from your host machine! Remember your host machine and virtual machine are two spate entities at this point but they share the same hardware of the host machine! Finish the installation of Ubuntu by selecting the options you like.

**Setting up your Linux system**

Before even installing Freesurfer we need to properly set up Linux first. After finishing the installation you will be greeted with some customization options of Ubuntu. Remember your username and password you have chosen! The first thing you need to do is click on the most top icon on you left screen. This icon will allow you to search through your virtual machine. Search for Terminal and after you found it lock it to your launcher so you don't have to look for it in future sessions. The first two commands you are going to type is **sudo apt-get update** and **sudo apt-get upgrade** in that order. These two commands will update and upgrade any package and library you have and yes those spaces are needed in the command! You will notice that every command that you use is very sensitive to syntax errors. If there are spaces in the commands of capital letters you need to add them otherwise you will get errors. This is the most common error so always check if you typed in the command correctly. After the updates and upgrades are finished close the virtual machine by selecting the option Power off the machine after pressing the X in the right corner. Restart your virtual machine and navigate to the Devices tab on the VirtualBox menu at the top of your screen. Select the option to Install Guest Additions CD image and restart the virtual machine. Now go to Machine tab on your VirtualBox menu at the top of your screen. Navigate to the tabs General and in General go to Advanced and select the option Bidirectional for both Shared Clipboard and Drag'n'Drop. Now you should be able to copy and paste commands from outside the virtual into your virtual machine! Now you don't have to type everything! The next thing is to grant yourself access to the files on your host machine, so you can transfer files for analysis from your host machine to your virtual machine. The first thing to do is to create a new folder on your host machine in an easy to reach place (Desktop) that becomes the shared folder between the host machine and virtual machine (give it an easy and short name!). After you have created this shared folder on your host machine we need to link it to the host machine. Power off your virtual machine and go to the settings options of your virtual machine by right clicking on it in your VirtualBox home screen. Navigate to the Shared Folders tab and press the plus sign and select your newly created shared folder. Additionally select the option make permanent and auto-mount. Restart your virtual machine and go to the files icon on your left screen. Press the computer file and navigate to the file called media. If everything went well there will be the shared folder that you made in your host machine but now sf_ is added to the name of the folder. Don't press it yet. In order to gain access to this folder on your virtual machine we will have to add ourselves to a vboxsf group. Use the command: **sudo adduser username vboxsf** Replace username with the username you have chosen. Now if you navigate back

to the sf_shared_folder you can access it and everything you put in this folder on your host machine will be visible here.

**X-server**

Before we move on to installing Freesurfer we need to set up one more thing. With the Linux operating system we can run applications through the command line but in order to run any GUIs for neuroimaging tools we will need a X-server for windows. The most popular is Xming. Go to here https://sourceforge.net/projects/xming/ and download/install it with default settings. Run the xming server and it will show up as an icon in the bottom right corner of your host machine. Now we need to link the X-server to the virtual machine and we can do this using the following command: **echo export DISPLAY=localhost:0.0" >> ~/.bashrc**

**Freesurfer 6.0**

Finally we have arrived at the Freesurfer part. The first thing is to download the most recent version of Freesurfer using the following command:
http://surfer.nmr.mgh.harvard.edu/pub/dist/freesurfer/6.0.0/freesurfer-Linux-centos6_x86_64-stable-pub-v6.0.0.tar.gz
if the most recent version has changed just change the numbers from 6.0.0 to the most recent version.
After the download is finished install Freesurfer 6.0 with the following command: **sudo tar -xzf freesurfer-Linux-centos6_x86_64-stable-pub-v6.0.0.tar.gz -C /usr/local/**
With this command you will install Freesurfer 6.0 in the folder /usr/local. Once this is completed we have to change the permission in this folder so we can edit and create files in the folder. Use this command: **sudo chmod -R 755 /usr/local/freesurfer**
You are almost there! The next step involves you registering with Freesurfer to obtain a licence.
Go to https://surfer.nmr.mgh.harvard.edu/registration.html and after the registration is completed you will receive a license in your email. Copy the contents between the lines that read 'cut here' and go back to your virtual machine and use the command: **sudo nano /usr/local/freesurfer/license.txt**
This command basically creates a text file in the Freesurfer folder and in this txt file you have to paste the license. Exit the txt file with ctrl+X and press y to accept and save it as licence.txt and finally hit enter. Now we need to add the Freesurfer path to our 'terminal home screen'. Important to realize is that Ubuntu is BASH based so any other commands that are not for BASH shells will not work!
Use the following commands:
**echo "export FREESURFER_HOME=/usr/local/freesurfer" >> ~/.bashrc**
**echo "source \$FREESURFER_HOME/SetUpFreeSurfer.sh" >> ~/.bashrc**
One more last thing is to use the command:
**sudo apt-get install build-essential libjpeg62 libxss1 libgomp1 tcsh**
This command will install the libraries that are most of the time missing from the general package that Freesurfer 6.0 needs. If you still miss libraries you can use the command: **sudo apt-get install <name-of-needed-file>**

**Final Check**

Hover over the Xming server and check if it says Server:0.0 and type in bash in the terminal of your virtual machine. You should see the following:

-------- freesurfer-Linux-centos6_x86_6.0 --------

Setting up environment for FreeSurfer/FS-FAST (and FSL)

FREESURFER_HOME  /usr/local/freesurfer

FSFAST_HOME  /usr/local/freesurfer/fsfast

FSF_OUTPUT_FORMAT nii.gz

SUBJECTS_DIR  /usr/local/freesurfer/subjects

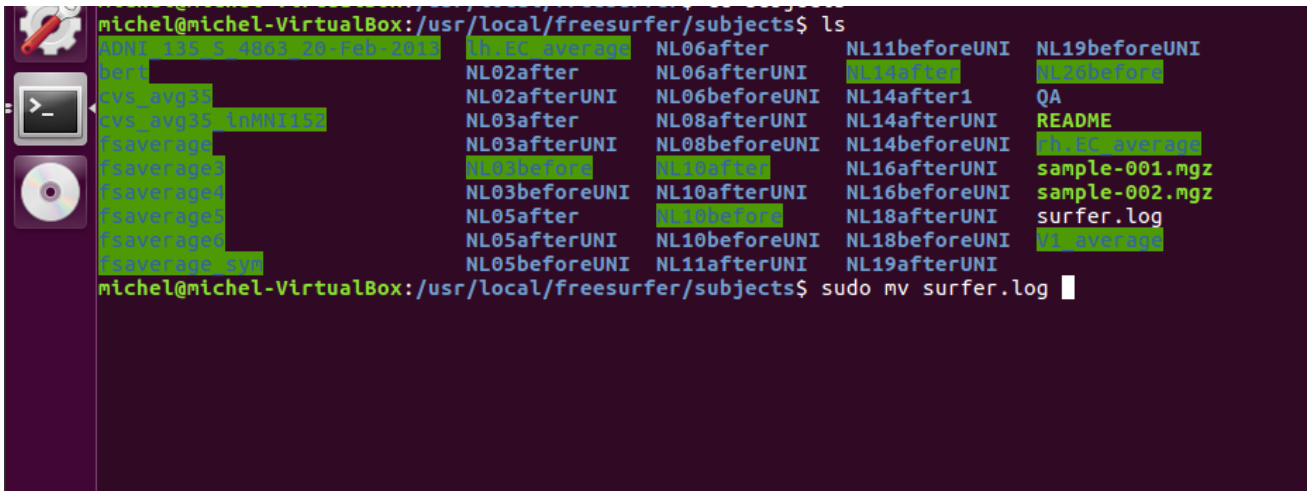
MNI_DIR  /usr/local/freesurfer/mni

If everything went smoothly you should test your installation by going to https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall#TestyourFreeSurferInstallation and use some of their examples.

**How to use Freesurfer 6.0**

Some simple but important commands
cd <foldername>
With this command you can change from folder to folder
So for example you want to go to the folder /usr/local/freesurfer because freesurfer is installed here. You can use cd /usr/local/freesurfer and you will be in the freesurfer folder.
Or if you already in the folder /usr/local and you want to move to the freesurfer folder you can just type in cd freesurfer and will do the same thing as cd /usr/local/freesurfer. The difference being that in the first case you were a folder that contained the Freesurfer folder whereas in the other situation you were in no folder at all. An extension of this command is cd ..
This allows you to go back to the previous folder you were in.

Another useful command is:
ls
With this command you can see what is inside a folder.
With these two command you can navigate through all your files and you will be using these two a lot.
You don't have to worry about making folders yourself as Freesurfer does this automatically for every subject you analyse.
sudo mv <foldername> <to where you want to move it>
important for this command to work is that you have to be in the folder that has the particular files you want to move. Otherwise you will also have to specify the folder where the file came from.

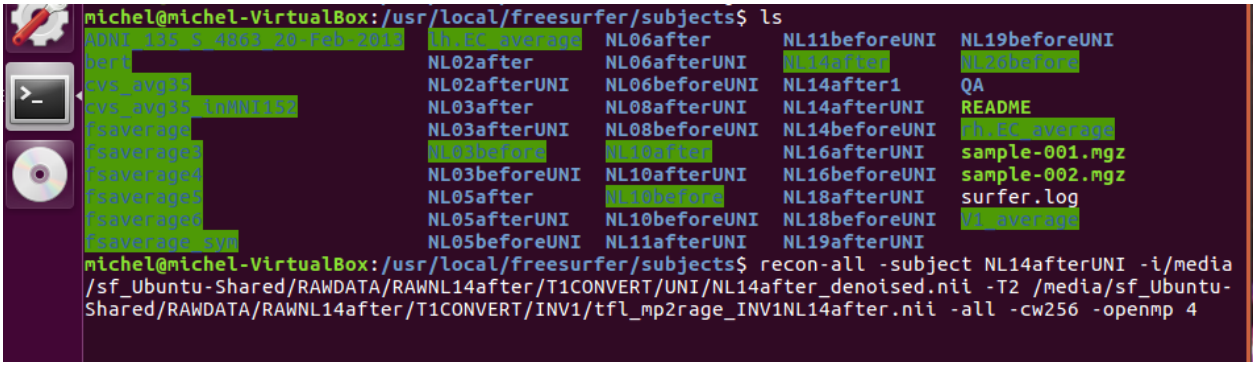



For example if I want to move the file surfer.log to my local folder while being in the same folder as where the file is located I can simply use sudo mv surfer.log /usr/local/ but if I was in a different folder I would have to use sudo mv /usr/local/freesurfer/surfer.log /usr/local/

In the case you want to remove something you can use the command:
sudo rm –r <foldername>
To make a new folder you can use:
mkdir <foldername>

And lastly if you want to open txt based files you can use the command :
cat <foldername>
For example if I wanted to open the surfer.log you can type cat surfer.log but you have to be inside the folder in which the surfer.log is located.

Freesurfer specific commands
Before you start to run the Freesurfer analyses make sure the data you want to analyse is located in the shared folder that you created earlier. Organize it nice and neatly for yourself so you can save yourself some time later on. Freesurfer's main command is called recon-all and this will most likely do everything you need but you can add some additional flags to the command to tailor it specifically to your dataset.
I will only focus on the volume based analysis and results but the recon-all command will give you surface based results. So before segmenting the substructures of the hippocampus I would recommend just doing the recon-all pipeline by itself to save time because as you will notice just one recon-all analysis will take between 8-14 hours depending on how much resources you allocated to the virtual machine. The general command that I used to run the recon-all pipeline is as followed:
recon-all –subject <subjectname> –i /path/to/input_volume –T2 /path/to/T2_volume –T2pial –all –cw256 –openmp 4
the majority of the command line should speak for itself but the reason why I added the last part is due to technical issues. The –cw256 part is to ensure that the data you input in the recon-all pipeline has a field of view of maximum 256 by 256 mm. anything above this Freesurfer will not accept. The openmp 4 part is just to speed the recon-all pipeline up a little bit. The flag allows Freesurfer to access more cores that are present on your computer. With the number 4 it will grant access to 4 cores and hopefully speed the process a little bit. If you input volume is part of a series than you only have to select one of the images in the series as Freesurfer will find the rest automatically. As you can see I also added T2 images to the analysis to improve the segmentation. You don't have to add it but if you have the data why not? It will only improve.

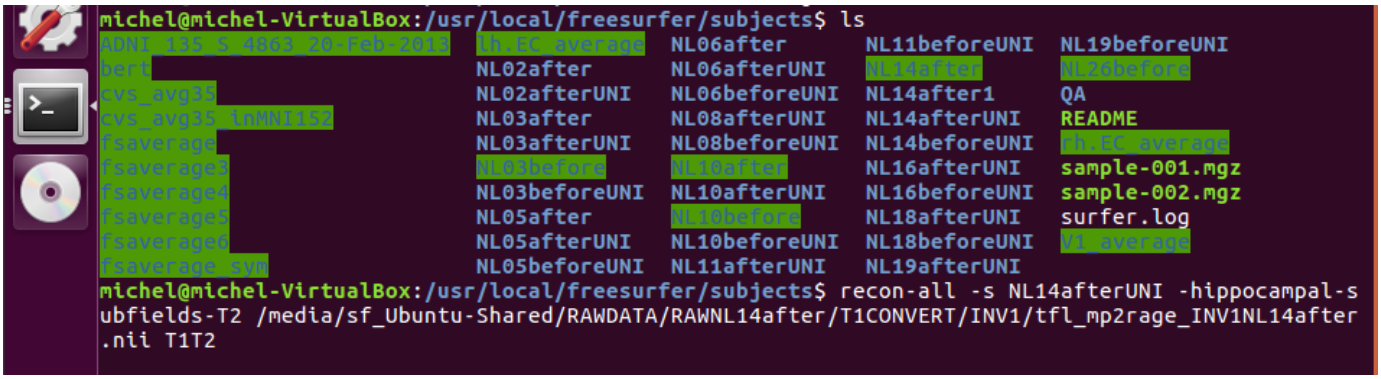


An example of the recon-all command line would be like the one depicted in the picture above. Very conveniently you don't have to make new folders as Freesurfer will make a new folder named the same as the subjectname and Freesurfer will place it in the folder /usr/local/freesurfer/subjects as you can see in the picture. Any results of the recon-all pipeline will be in this folder and error messages as well. As you can see the lines can get really long depending on how many folders you have and it is therefore of importance to correctly structure your data and make things easier for yourself!

**Hippocampal Subfields**

If everything went correctly, Freesurfer should say that it finished without any errors.
However you will still need to check for errors manually to ensure everything went well.
We will come back to the errors and how to check for them. If we assume everything went well and the segmentation looks really good you can put the results into the hippocampal subfields analysis of Freesurfer 6.0. This command is recon-all –s <subjectname> –hippocampal-subfields-T2 <file name of additional scan> <analysisID>
This command will once again combine T2 image data with T1 image data for better segmentation of the substructures in the hippocampus. The analysisID is basically an additional tag for yourself so you can easily distinguish it from other results. Important is that you use the same subject name that you created for the recon-all pipeline.

An example for the command is depicted in the picture above and as you can see I added the ID T1T2 so I know that these results were the results of a combination of T1 and T2 data. All the results will have this ID in the name.




**Errors**

There are two types of errors in Freesurfer: hard and soft errors.
Hard errors happen when the recon-all doesn't finish at all and a soft error happens after a recon-all pipeline did finish but when you are manually checking it you discover that something still went wrong. Therefore it is important to always manually check if there are any soft errors. Since they come in many variations I will refer you to their amazing site on what kind there are and possible solutions. https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TroubleshootingData

If there are any other problems I would recommend to try and google it and otherwise there is a mailing list of Freesurfer which you have to subscribe to but you can ask questions directly to the Freesurfer creators. Good Luck!