2016•2017
FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

The relationship between GABA neurotransmission and motor behavior in advancing age

Promotor :
dr. Koen CUYPERS

Copromotor :
Prof. dr. Raf MEESEN

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Mevr. LIZE HERMANS

Jo Van Keer-Van Mol

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

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Furthermore, I would like to thank all the subjects for their willingness to participate in the present study. Without their consistency, motivation and endeavor, completing this master thesis was not possible. Moreover, I would like to thank my parents and brother, for giving continuous support throughout the period of constructing this master thesis.

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Research context

Aging is an important subject worldwide. The influence on socio-economic levels is undeniable. Generally, older adults are more frail and thus more susceptible for common pathologies and comorbidities, this leads to an increase in medicals costs and hospitalization. Another consequence linked to this frailty of a graying population worldwide, is the decline in employment rates, negatively influencing the economy in the inhabited state/country (A. D. S. e. E. I. FOD Economie, 2011).

The topic of this master thesis is situated in the neurological branch of Physiotherapy and Rehabilitation Sciences. The specific aim of this master thesis was to measure GABA neurotransmission in relation to motor inhibition and behavioral relevance, with advancing age. The assessment of the GABA neurotransmission, linked with mean reaction time and stop signal reaction time (SSRT), helped to achieve a better understanding in the behavioral relevance of GABA neurotransmission in the healthy aging population. Magnetic Resonance Spectroscopy (MRS) has become an interesting and effective tool to measure in vivo GABA-concentrations in distinct brain regions reliably in healthy aging subjects (Gao et al., 2013). Many studies exist using MRS in the assessment of GABA-concentration in the healthy aging brain (Gao et al., 2013). In addition to this, transcranial magnetic stimulation (TMS) studies do also exist in a big majority (Heise et al., 2013). However, studies combining both techniques are scarce and much more research to the behavioral relevance of these GABA-concentration alterations, with advancing age, needs to be executed. Because the lack of consistent findings considering these behavioral aspects, the following research question was proposed: “Is there a decrease in mean reaction time and an increase in the latency of the reaction to the stop signal (SSRT) with a decreased GABA-concentration in elderly as compared to their younger counterparts?”. In that manner, the work currently presented, tries to provide a better understanding in the behavioral relevance concerning motor activity in healthy human aging.

There was no contribution of the student to the method and research protocol, because the research itself was ongoing. Furthermore, the recruitment and data-acquisition were all

completed, thus no contribution of the student was included. Considering the processing of statistics on the acquired data, the student managed to independently execute the adequate statistical procedures. The academic writing process was mainly conducted by the student, although the promotor and co-promotor of this master thesis offered help and guidance to their best ability.

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1 Abstract

Background

Over the last 20 years, the average life expectancy in Belgium increased with 4.4%. Motor functioning and aging are associated in a way that aging results in motor functioning declines. Gamma aminobutyric acid (GABA) is the paramount inhibitory neurotransmitter in the human brain. Hence, it is hypothesized that: 1) GABA-concentration in the brain decreases with advancing age 2) Mean reaction time decreases with advancing age and 3) stop signal reaction time (SSRT) increases with advancing age. This work will explore the aforementioned hypotheses and the underlying affinity of the variables concerning these hypotheses.

Objectives

The present work investigated the effect of aging on GABA-concentration and the concurrent relationships between GABA-concentration, SSRT and mean reaction time.

Participants

29 older (<74 years of age) adults and 24 younger (<30 years of age) adults participated in this study. Female and male sexes were both included. Subjects were right handed and healthy as assessed by a thorough screening tool (cfr. infra in appendix).

Measurements

Magnetic Resonance Spectroscopy (MRS), more specifically a MEGA-PRESS sequence was used to assess the regional GABA-concentration in the brain region of interest, the motor handknob. Mean reaction time and SSRT were measured in milliseconds (ms), when conducting a go/no go task.

Results

No significant difference in GABA between age-groups was found, nor did any significant difference occur considering mean reaction time, between young and old. Concerning SSRT a significant difference (p=.0004) was found between younger (231.910 $\pm$ 22.410 ms) and older adults (251.888 $\pm$ 15.617 ms), older adults show higher SSRTs in comparison to their younger counterparts. Ultimately, no significant correlations between aforementioned variables were found.

Conclusion

Remarkably, the current study shows no difference in GABA-concentrations comparing both age groups, although extensive research has proven otherwise (Gao et al., 2013). However, this result should be interpreted carefully, because of the small sample size in this work.

2 Introduction

Worldwide aging is a hot topic especially in developed countries. Aging has an important impact on a socio-economic level, as well as on an individual level. More specific, in Belgium, the average life expectancy evolved from 76.7 years (1994) to 81.1 years (2014). This reflects an increase in life expectancy of 4.4% over 20 years (S. B. FOD Economie, 2015). Furthermore, in 2000, 16.75% of the Belgian population consisted of older adults (age: 65+). In 2060, this population is estimated to consist of 24.75% of the Belgian population (A. D. S. e. E. I. FOD Economie, 2011).

Aging is a process in which a decline in motor skills is found. The decline is accompanied by structural and functional changes in different regions of the brain (Fling & Seidler, 2012; Heuninckx, Wenderoth, & Swinnen, 2008; Langan et al., 2010; Nielson, Langenecker, & Garavan, 2002; O'Sullivan et al., 2001; Seidler et al., 2010; Turner & Spreng, 2012). One of those regions includes the frontal lobe in which processing of motor functioning, and motor inhibition in particular, takes place. This lobe is part of the inhibitory network consisting of the prefrontal cortex, inferior frontal cortex, the right presupplementary motor area and the subthalamic nucleus (van den Wildenberg et al., 2010). The process of motor inhibition influences the motor performance and therefore the motor task (Stagg, Bachtiar, & Johansen-Berg, 2011). Motor inhibition can be described as the possibility to stop or reduce an ongoing voluntary motor activity in adaptation to the requirements of the task or to environmental changes (Verbruggen & Logan, 2009). Hence, when the brain is subjected to age-related structural changes, motor functioning can decline as well (Jordan & Rabbitt, 1977). With advancing age, people will experience longer reaction times (Antosiak-Iwanska et al., 2009; Cuypers et al., 2013; Fujiyama et al., 2012; Fujiyama, Tandonnet, & Summers, 2011), and impairments in motor functioning in general (Calautti, Serrati, & Baron, 2001).

One of the important contributors considering motor inhibition in the human brain is the neurotransmitter gamma aminobutyric acid (GABA)(McCormick, 1989). GABA is the major inhibitory neurotransmitter in the human brain and central nervous system which prevents excessive neural and motor activity in muscles (Lundy-Ekman, 2013). GABA is derived from

glutamate (the most important excitatory neurontransmitter in the human brain) by glutamic acid decarboxylase (GAD) within GABAergic neurons and is metabolised to succinic acid semialdehyde by GABA transaminase (GABA-T) and is then transformed to succinate, primarily within astrocytic mitochondria (Chang, Cloak, & Ernst, 2003). In this work in vivo proton Magnetic Resonance Spectroscopy (H-MRS) will be used to measure GABA-concentration in the frontal lobe during a behavioral task. Noteworthy, recent studies show a decline in GABA-concentration with aging (Gao et al., 2013). Recently, a cross-sectional study of adults (20–76 years of age) indicated an age-related decline in GABA-concentration in the frontal cortex of 5% per decade starting from adolescence (Gao et al., 2013). One interesting MRS-protocol specifically, the Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS), is a sequence that allows rapid and reliable quantification of GABA-concentrations in the brain (Cai et al., 2012; Gomez et al., 2012), and is going to be used in this work to measure the in vivo GABA-concentrations. MEGA-PRESS enables direct regional investigation of GABA by taking advantage of the known scalar couplings within the GABA molecule (Antosiak-Iwanska et al., 2009; Edden & Barker, 2007; Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998; Mullins, McGonigle, O'Gorman, Puts, Vidyasagar, Evans, Cardiff Symposium on, et al., 2014). The Scalar coupling is the existence of the interaction between hydrogen nuclei within a given molecule, transmitted through the bonding electron network. The aforementioned interaction, changes the appearance of the spectrum and the time evolution considering the spins of the hydrogen nuclei. The application of a radiofrequency pulse to one coupled spin could lead to a modification of the time evolution of the coupling partner and the appearance of the corresponding peak within the spectrum (Mullins, McGonigle, O'Gorman, Puts, Vidyasagar, Evans, Cardiff Symposium on, et al., 2014) . Thus far, MEGA-PRESS has been able to prove that GABA-concentrations correlate with cerebral blood flow as well (Donahue, Near, Blicher, & Jezzard, 2010). Moreover, several MRS studies have investigated the role of GABA in motor and sensory functioning in healthy populations (Porges et al., 2017). In addition to this, MRS is a powerful tool when age-related changes in brain chemistry have to be measured (Chang, Ernst, Poland, & Jenden, 1996; Charles et al., 1994; Christiansen, Toft, Larsson, Stubgaard, & Henriksen, 1993; Cohen et al., 1995; Fukuzako et al., 1997; Kreis, Ernst, & Ross, 1993; Lim & Spielman, 1997; Novotny, Hyder, Shevell, & Rothman, 1999; Peden et al., 1990; Pfefferbaum, Adalsteinsson, Spielman, Sullivan, & Lim, 1999; Saunders, Howe, van den Boogaart, Griffiths, & Brown, 1999; Toft, Christiansen, Pryds, Lou, & Henriksen, 1994). MRS has proven efficacy in

measuring the age-related changes in neurotransmitter concentration of GABA and glutamate in the dorsolateral prefrontal cortex (Grachev & Apkarian, 2001). More specifically, studies considering MRS, where comparisons between younger and older adults were made, show age-related changes considering the GABA-concentration, N-acetyl Aspartate (NAA), Choline (Cho) and Creatine (Cr) in the human brain (Chang et al., 1996; Charles et al., 1994; Christiansen et al., 1993; Cohen et al., 1995; Fukuzako et al., 1997; Lim & Spielman, 1997; Novotny et al., 1999). Furthermore, MRS measures metabolite concentrations, with sufficient sensitivity to detect individual differences. To date, there are several studies investigating different brain regions and their correlated GABA levels. However, GABA levels were not correlated between the different brain regions as found by Boy et al., 2010 and Grachev and Apkarian., 2000, 2001 (Boy et al., 2010; Grachev & Apkarian, 2000, 2001).

Noteworthy, the measurement of intra-cerebral GABA via MRS is hindered because the resonances of other metabolites such as Glutamate (Glu), NAA, Cr, Homocarnosine and other macromolecules overlap the spectrum. Cr for example has co-resonant metabolite peaks around 3.01 ppm (Waddell, Avison, Joers, & Gore, 2007), making it difficult to differentiate GABA individually in the given spectrum (Antosiak-Iwanska et al., 2009; Govindaraju, Young, & Maudsley, 2000; Waddell et al., 2007). Although, it is possible to measure GABA-concentration with MRS by tailoring the MRS experiment explicitly to isolate the GABA-related signals from the spectrum (Puts & Edden, 2012). Conclusively, among a wide variety of methods for the measurement of GABA, the only technique allowing a direct, non-invasive investigation of endogenous GABA in vivo, is MRS (Puts & Edden, 2012).

The behavioral task consists of a go/no go task, more information about this specific procedure is described in the method section. In addition to this, magnetic resonance spectroscopy (MRS) will be used to measure the concentration of GABA in the aging human brain in the primary motor cortex (M1) and more specifically the motor hand-knob, our region of interest (Stagg, 2014).

Secondly, the integrity of the GABA receptors can be assessed using an intracortical motor inhibition protocol (Heise et al., 2013; Opie & Semmler, 2014). Consequently, motor inhibition

can be assessed using transcranial magnetic stimulation (TMS). TMS is an excellent technique to investigate the neurophysiological changes occurring in the aging brain tissue (Levin, Fujiyama, Boisgontier, Swinnen, & Summers, 2014). More specifically, it is a technique in which a magnetic coil is used to generate a magnetic field. This magnetic field can generate a local electric current at the stimulated brain site. As a result of this electrical current, the blood flow and metabolism in the stimulated brain tissue will be altered (Strafella & Paus, 2001). Furthermore, when sufficient electrical current is induced in the brain, a motor evoked potential (MEP) can be evoked. An MEP is the electrical response evoked in a muscle or motor nerve by electrical or magnetic stimulation on the human motor cortex (Pubmed, 1996). When the electrical current is induced, the MEP will follow the corticospinal tract down to the spinal cord, into the peripheral nerve and the neuromuscular junction. The MEP is the net effect of the inhibitory and excitatory impulses in the motor cortex and can develop a muscle contraction (Levin et al., 2014). The muscle contraction can be visualized and the MEP can be measured by electromyography (EMG). In order to generate an MEP, the given stimulus induced from the magnetic field needs to be powerful enough. The desired stimulus intensity in mV, to reach an MEP, is different across individuals. When the stimulus intensity is strong enough to produce an MEP, it is called a suprathreshold stimulus, in the contrary when the stimulus is insufficient to produce an MEP it is called a subthreshold stimulus (Di Lazzaro et al., 2009).

To better understand the process of motor inhibition on the intracortical level, it is important to describe the concept of single-and paired-pulse TMS-protocols. Hence, when a single pulse TMS protocol (fig 1) is used, with sufficient intensity, an MEP will appear.

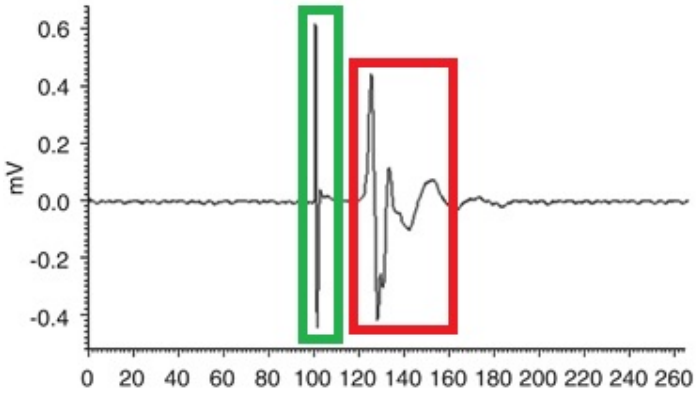


Figure 1: Single pulse TMS. Green: suprathreshold stimulus artefact induced by the discharge of the TMS coil**.** Red: MEP**.** (Fitzgerald, Williams, & Daskalakis, 2009)

Moreover, when a paired-pulse TMS technique is used, two pulses are initiated at different time intervals. Two different time protocols relying on different intervals are commonly used: 1) short interval intracortical inhibition (SICI) and 2) long interval intracortical inhibition (LICI). SICI and LICI correspond to GABAa (Ilic et al., 2002; Ziemann, Lonnecker, Steinhoff, & Paulus, 1996a) and GABAb (McDonnell, Orekhov, & Ziemann, 2006; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999) postsynaptic receptor activity respectively. SICI is a paired pulse TMS technique (fig 2) where a subthreshold conditioning stimulus precedes a supratreshold test stimulus with an interval of 1-5ms, whereas for LICI a supratreshold conditional stimulus precedes a supratreshold test stimulus with an interval of 100-150 ms (Kujirai et al., 1993; Ziemann, Lonnecker, Steinhoff, & Paulus, 1996b).

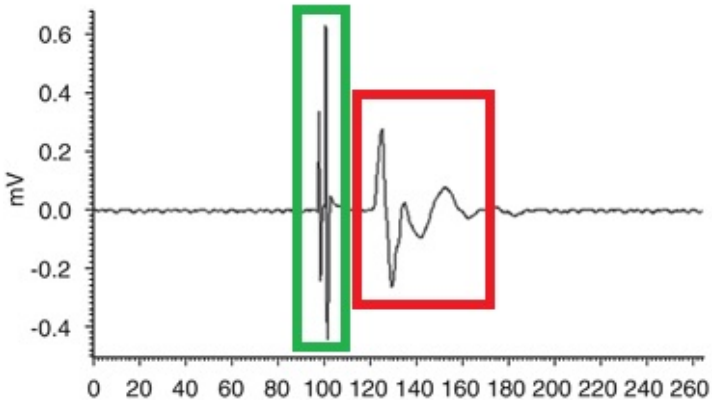


Figure 2: Paired-pulse TMS. Green: first pulse: subthreshold stimulus or suprathreshold stimulus (SICI or LICI), second pulse: suprathreshold stimulus, space between both pulses: interstimulus interval (ISI). Both pulses are artefacts induced by the discharge of the TMS coil. Red: MEP. (Fitzgerald et al., 2009)

Additionally, aging results in a decrease of SICI, leading to an increase of the average MEP with 1.6% per year of age, starting from the age of 20. Moreover, when movement preparation is considered, a decrease in SICI is measured. The decrease also starts from the age of 20 years and declines with ±.3% per added year of age (Heise et al., 2013). Likewise, during a motor task an indirect measure of successful motor inhibition is the cortical silent period (CSP). CSP is the period after a successful MEP. In this period all EMG activity is suppressed. Following this period of suppression all EMG activity normalises. The duration of this CSP is related to the GABAb-ergic activity and is negatively correlated to it (Benwell, Mastaglia, & Thickbroom, 2007; Levin et al., 2014; Opie & Semmler, 2014; Poston, Kukke, Paine, Francis, & Hallett, 2012).

Hence, for a successful inhibition during a go/no go task, in the early stage, following the go signal, the CSP will decrease, whereas the LICI will increase. In the later stage, following the go signal, the CSP will increase in which LICI will decrease. As mentioned above the decrease in CSP is concurrent with an increase of LICI and represents GABAb-ergic activity. This leads to a reduction in corticospinal excitability, at least if no choices between alternative responses have to be made (van den Wildenberg et al., 2010).

To study inhibitory functioning a specific task was applied, namely a go/no go task (Verbruggen & Logan, 2009). Commonly, in a go/no go task, the subjects need to perform a speeded response to a visual stimulus, when the go/no go task is considered there are two options: 1) the signal given to the test subject needs to be responded to by means of motor activity, described as the go response or 2) occasionally, the go signal is followed by a no go signal, where subjects need to withhold their response, described as the no go response (Verbruggen & Logan, 2009). Thus, when such a go/no go task is executed generally the go signal will be followed by a no go signal, the process that is finished first, determines whether the task is executed or inhibited, this is formally known as the independent horse-race model (Verbruggen & Logan, 2009). Therefore, if the stop process initiated by the stop signal finishes first, the task will be inhibited. Whereas, if the go signal wins the race, the task will be executed (van den Wildenberg et al., 2010).

As follows, (mean) reaction time is a variable that has an important role in inhibitory motor control. Reaction time is the measurement of the time course of excitability changes, of the motor system when a manual response is produced (van den Wildenberg et al., 2010).
Moreover, Stagg, Bachtiar et al., 2011, conducted an experiment in which they found a positive correlation between GABA-concentration and mean reaction time (Stagg, Bachtiar, et al., 2011). In respect to the above mentioned knowledge, it is possible to measure the latency of the stop process, the stop signal reaction time (SSRT), which serves as a surrogate marker of the inhibitory efficiency. The go/no go task has a key role in the assessment of SSRT and is becoming a golden standard in the assessment of inhibitory efficiency. As follows SSRT is most representative for daily activities involving response inhibition (van den Wildenberg et al., 2010). The prefrontal cortex, alongside with the right inferior frontal cortex, the right

presupplementary motor area and the subthalamic nucleus play a key role in stop-signal inhibition during the go/no go task (van den Wildenberg et al., 2010). The aforementioned brain areas are crucial in the planning of motor activity and essential in successful response inhibition. However, M1 is the determinative cortical site where the final motor plan will be executed or inhibited (Picazio et al., 2014).

Because many inconsistencies regarding differences between younger and older adults exist in motor inhibition, this work is focused specifically on the inhibitory motor activity. This short introduction leads to the hypothesis of this work: “There is a decrease in mean reaction time and an increase in the latency of the reaction to the stop signal (SSRT) with a decreased GABA-concentration in elderly as compared to their younger counterparts.” In which the importance of the comparison to the younger counterparts is reflected and where there is a tendency to illustrate aging as a continuum. The evolution of mean reaction time and SSRT in aging will be accounted for and will be examined thoroughly. Remarkably, few studies conducted concerning the subject, motor inhibition, use a combination of Transcranial Magnetic Stimulation (TMS) and MRS to measure motor inhibition as such. Studies where only TMS is used are larger in numbers. In this study MRS is chosen as the technique to measure GABA-concentration, with the downside that MRS can not specify between GABAa and GABAb synaptic activity, as is possible with a TMS protocol (Stagg, Bestmann, et al., 2011; Tremblay et al., 2013). However, an advantage of MRS is the measurement of the GABA-concentration more specifically at the brain region of interest, which is beneficial in comparison to the TMS-measures, in which more inaccuracies arise. In addition to this, MRS is less invasive compared to TMS-measures (Hone-Blanchet et al., 2015) .

3 Method

3.1 Participants

29 older (<74 years of age) adults and 24 younger (<30 years of age) adults participated in this study. Female and male sexes were both included. Subjects were right handed as indicated by the Edinbrugh Handness inventory (Appendix 1). All participants were in a healthy state as determined from questionnaires administered during a screening session, the used screening and training protocol were different for younger (appendix 2) and older (appendix 3) adults. The in–and exclusion criteria of the subjects are displayed in the following table:

Inclusion criteria	**Exclusion criteria**
• Right-handed (Edinburgh Handedness Inventory (mean ± SD: 91 ± 14) • No Neurological and/or psychiatric conditions • No drug use/abuse • No use of psychoactive medication • No abnormalities in sleep pattern • No depression • No reduced visual acuity (Appendix x) • No subjects with non-removable ferromagnetic objects	• Left-handed • Neurological and/or psychiatric conditions • Drug use • Use of psychoactive medication • Abnormalities in sleep pattern • Depression • Reduced visual acuity • Subjects with non-removable ferromagnetic objects

3.2 Procedure

3.2.1 Primary outcome measures

The primary outcome measure is GABA-concentration as measured by the MEGA-PRESS sequence MRS-technique, at the primary motor cortex (M1), more specifically the motor handknob, on the left side and in the occipital cortex.

3.2.2 Secondary outcome measures

The secondary outcome measures were the different variants of the reaction time including: stop signal reaction time (SSRT), this is the latency of the response to the stop signal, and mean reaction time (Verbruggen & Logan, 2009).

3.2.3 Magnetic Resonance Spectroscopy

A medical screening was applied before the patients were exposed to the measurement and the inhibition task. The participants payed 3 visits to the research institute. Day 1: visit for the screening, day 2: a few weeks later for the MRS-measurement (each MRS measurement takes approximately 10 minutes) and day 3: for the stop-signal task. MRS-measurement and the stop-signal task are executed separately.

During this study Magnetic Resonance Spectroscopy (MRS) was the procedure of choice to measure the GABA-concentrations in the primary motor cortex (M1) and the occipital cortex. The specific protocol for the MRS-measurement is a MEGA-PRESS sequence, this sequence will be explained in the following paragraph. To measure the GABA-concentrations in well-defined brain areas, voxels were utilized. Voxels are small 3D-boxes which pinpoint the location of the specific brain region in which the GABA-concentration needs to be measured. These voxels are placed in the left primary motor cortex and in the occipital cortex. Before the voxel is administered, a T1 anatomical image is made of the brain to make sure the voxel is placed on the adequate anatomical landmark.

3.2.4 MEGA-PRESS sequence

To measure the GABA-concentration in vivo, the MEGA-PRESS sequence, a specific MRS-technique was the method of choice. This created the possibility to differentiate GABA signals from stronger overlying signals of different metabolites by knowing the scalar couplings within the GABA molecule. The Scalar coupling is an interaction between different hydrogen nuclei within a molecule, this interaction is transmitted via the bonding electron network, which alters the appearance of the spectrum and time-evolution of spins (couplings) during an experiment. If a radiofrequency pulse is applied to a coupled spin, the time-evolution of a coupling partner is modified and the appearance in the peak of the spectrum is changed. To measure GABA, an edited pulse at 1.9ppm GABA spins is applied to focus on the evolution and spectrum on 3 ppm GABA spins. This is referred to as an ON-spectrum. In the OFF-spectrum an inversion pulse of the ON-spectrum is applied. By extracting the ON-spectrum frequencies, the 3 ppm GABA spins are visible as well as the other metabolites, whereas if the OFF-spectrum frequencies are subtracted from the ON-spectrum (fig 3), only the 3 ppm GABA spins peak frequencies are visible providing a measure of GABA-concentration (Mullins, McGonigle, O'Gorman, Puts, Vidyasagar, Evans, & Edden, 2014).

All images were collected on a Phillips 3T achieva Magnetic Resonance scanner, with a 32 channel receiver head coil located at Academic Hospital Gasthuisberg, Leuven, Belgium. Volunteers were lying in a supine position head first in the scanner. T1 weighted anatomical images (fig 4) were collected with a Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence with TR=9.6 ms, TE=4.6 ms and a flip angle of 8º as parameter settings. The data consisted of 160 slices covering the brain. Slices were 1.2 mm thick, with no gap between the slices. Voxel size was 0.98x0.98x1.2 mm in a 256x256 matrix with a 250 mm^2 field of view (FOV).

The above mentioned methods were used to measure our primary outcome measures as accurately as possible, although limitations are present. The limitations of MRS in general are discussed in the introduction part of this work.

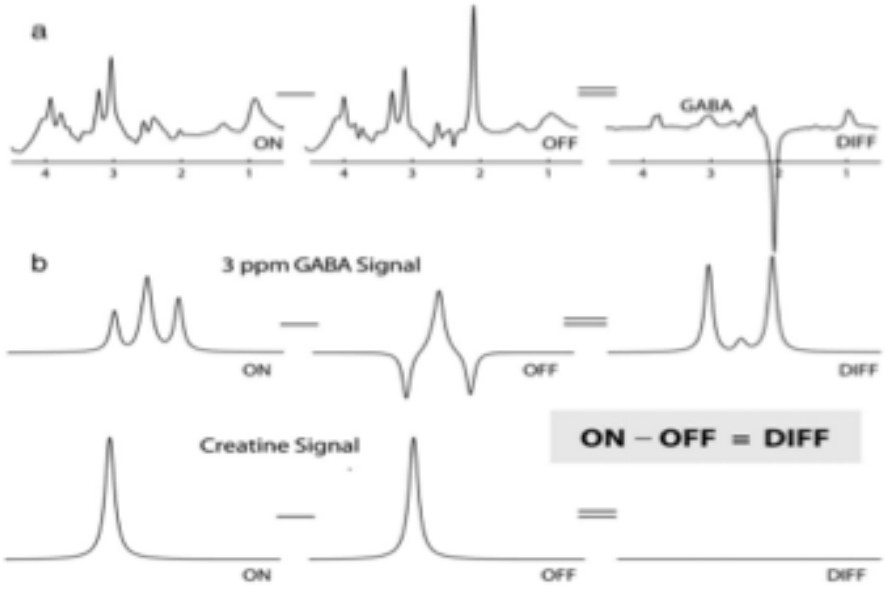
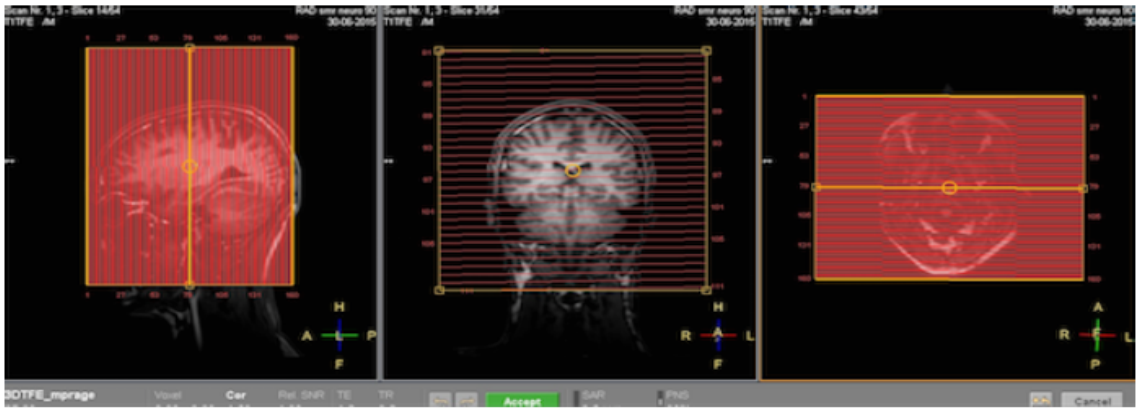
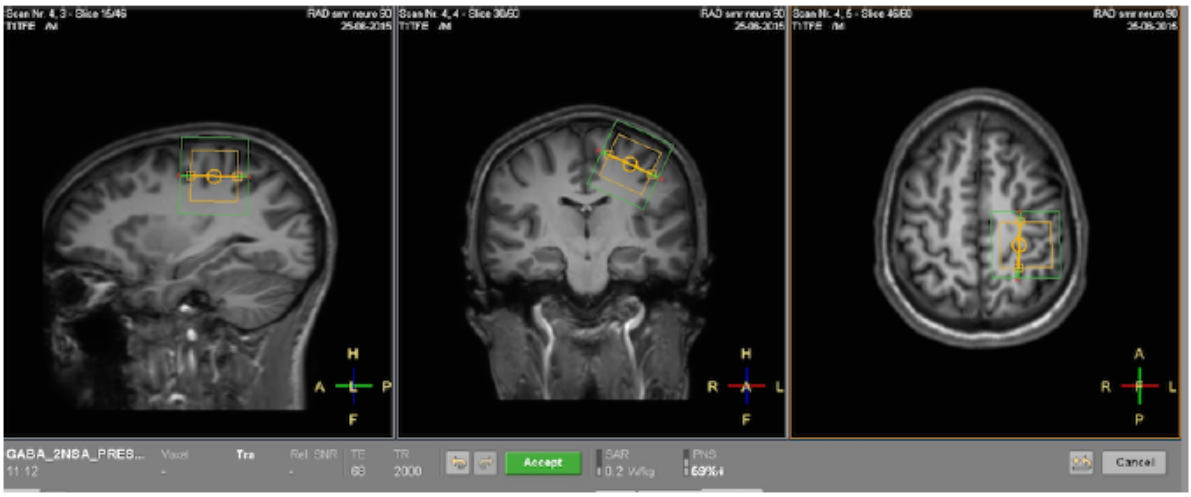


Fig. 1. Schematic diagram of MEGA-PRESS editing for GABA. (a) Editing pulses applied at 1.9 ppm modulate the shape of the GABA signals at 3 ppm (b). Subtracting scans acquired without these pulses (labeled OFF) from scans acquired with the editing pulses (ON) removes overlying creatine signals from the edited spectrum, revealing the GABA signal in the difference spectrum (labeled DIFF). (b) shows the effect of editing pulses on signals at 3 ppm only.

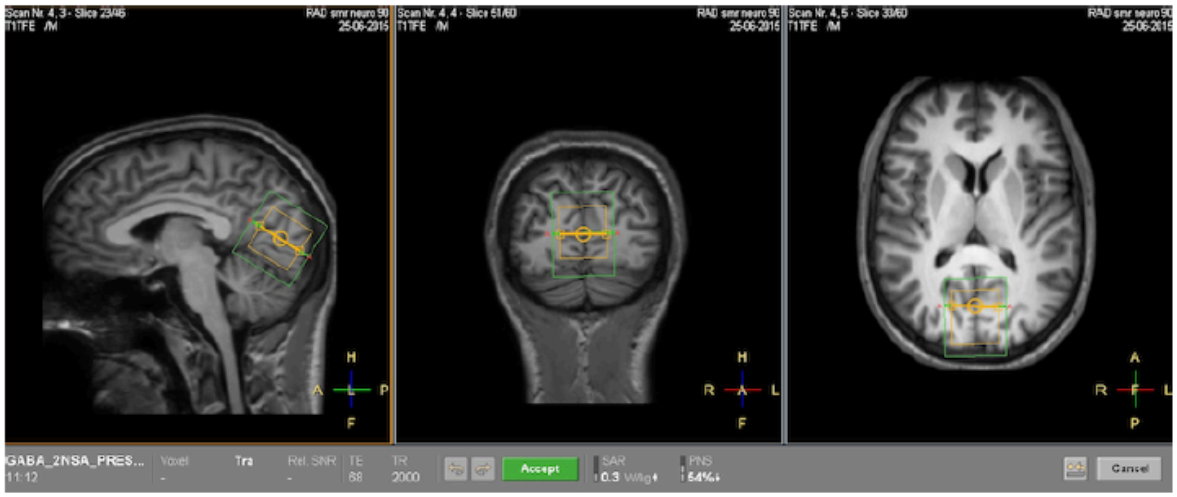
Figure 3: MEGA PRESS on/off spectrum (Mullins, McGonigle, O'Gorman, Puts, Vidyasagar, Evans, & Edden, 2014)

Rotate the box, in a way you can capture the brain.





Set the voxel over the hand knob region at M1. First: load in the T1 scan. Second: Set the voxel. Drag the box up in the sagittal view (left). Scroll through the axial view (right) to see the box. Put the box on the omega shape. Go back to the sagittal view and rotate the box parallel to the skull (box should be centered over a hook shape -if visible-. Only the brain should be inside the inside (orange) box without the skull. Go to the coronal view and rotate the box parallel to the skull. Finally check in the axial view if the box is approx. in the middle of your slices. For example if you have 15 slices (count how many orange boxes you see when scrolling through the axial image). The omega should be visible at slice 8 (7 of 9 should be fine too).



Go to the coronal view (middle) and place the box in the middle of the brain. Make sure the box is rotated in a way that it is straight. Axial view (right): Make sure the box is straight and drag it to the back of the brain. Set the box in the sagittal view (left) and rotate the orange box parallel to the cerebellum. Make sure only brain is in the box.

Figure 4: protocol for voxel localisation, T1 weighted anatomical images.

3.2.5 Stop-signal paradigm - go/no go task

Participants performed an anticipated version of the stop-signal task (fig 5). This task measured the ability to cancel a prepared movement. On a computer screen, a vertical indicator will be presented moving upwards at an equal and constant rate on each trial, crossing a horizontal target line at 800 ms from onset. The main task is to release a pressure sensitive plate by lifting the right hand when the indicator has reached the red target line, this is allocated as the go task. The go task will be measured as the time that is needed to lift the hand off the pressure sensitive plate, starting from the go signal, namely the reaction time. In the stop trials the indicator will stop before reaching the target line, indicating that participants need to cancel the movement of lifting their hand (fig 6)(Coxon et al., 2016; Coxon, Van Impe, Wenderoth, & Swinnen, 2012). Unlike the latency measured in a mean reaction time task, it is impossible to measure the latency to a stop response directly. The horse-race model is used to estimate the latency to the stop response (SSRT). Thus, the stop signal paradigm can be described as an independent horse race model, where figuratively there is a race between a go process and a stop process. The go process is triggered by the appearance of a go signal. The stop process is triggered by the presentation of a stop signal. When the stop process is finished before the go process, response inhibition was allocated as successful and no movement is executed. When the go process is finished before the stop process, response inhibition was allocated as unsuccessful and movement will occur. SSRT will be measured as the latency of the stop process (Verbruggen & Logan, 2009). More specifically, SSRT can be calculated by subtracting the mean stop-signal delay from the go Reaction Time. The mean stop-signal delay, is the delay of presentation of the stop signal, right after the go signal is presented (Colzato, van den Wildenberg, van Wouwe, Pannebakker, & Hommel, 2009) (fig 7). The stop signal paradigm, is widely used to measure response inhibition (Verbruggen & Logan, 2009). One of the limitations of the go/no go task is the amount of concentration a specific subject has. The concentration of the subject can depend on several factors such as distraction and the cognition of the subject (Verbruggen & Logan, 2009), these factors were taken into consideration in the medical screening as well as in the exclusion criteria. A dynamic staircase algorithm was used to adjust the time that the indicator stops in stop trials, ensuring equal numbers of successful and unsuccessful stop trials.

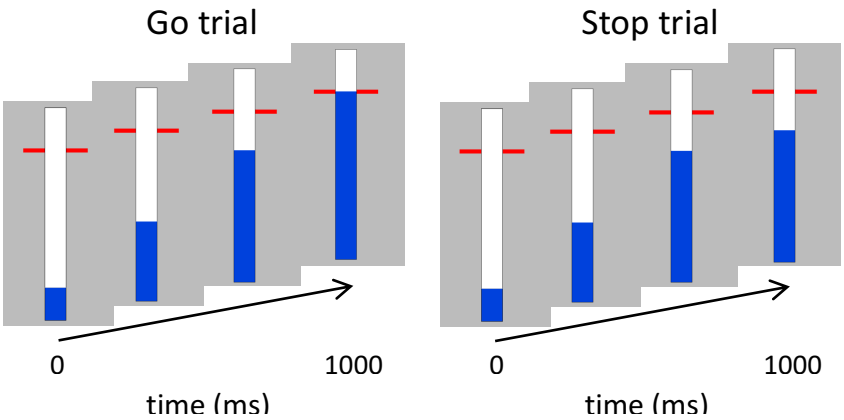


Figure 5: Go trial and stop trial (Coxon et al., 2016; Coxon et al., 2012)

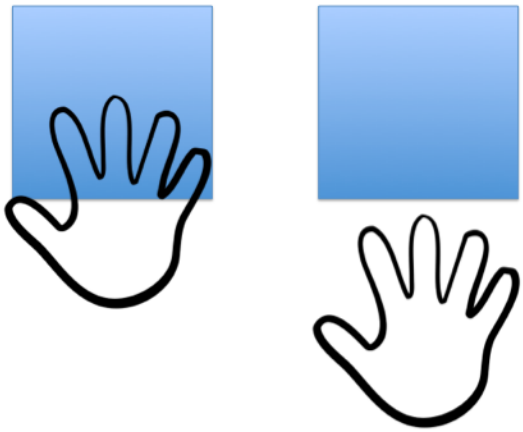


Figure 6: Hand on and off pressure plate

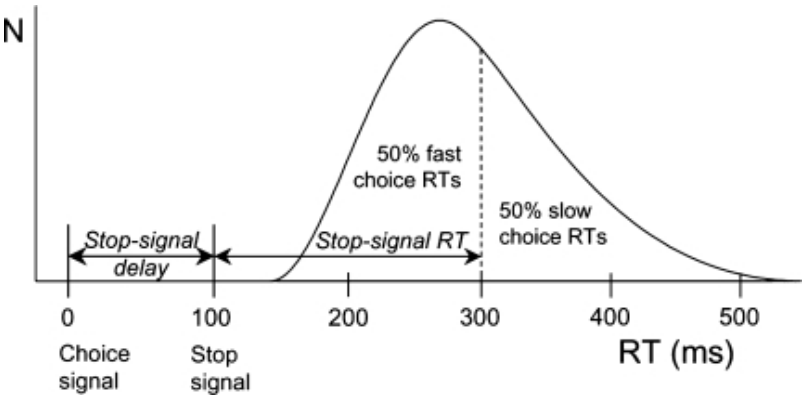


Figure 7: calculation of SSRT according to an independent horse race model. A representation to clearify the exact measurement of the stop-signal RT (SSRT). In this example the SSRT would measure 200 ms, given that

the delay of the stop signal to the go signal is 100ms and the mean reaction time is 300 ms (Colzato et al., 2009).

3.2.6 Data-analysis

The data was analyzed with JMP. A cross-sectional study design was applied at the subjects. A linear regression model was the method of choice to correlate the primary outcome measures and the secondary outcome measures, to see a linear relationship between the different variables. Moreover, a Wilcoxon Signed Rank-test was conducted to measure the relationships between 2 independent age groups.

3.2.7 Statistical analysis

3.2.7.1 Primary outcome measures

A student's T-test was utilized to measure the relationship between the GABA-concentration in the different age groups. A p-value ≤ 0.05 was considered significant.

3.2.7.2 Secondary outcome measures

The statistical analysis of the secondary outcome measures was conducted by using JMP. The intention of the statistics was to find a correlation between the GABA-concentration and mean reaction time at first hand and a correlation between the GABA-concentration and SSRT at second hand. Because SSRT and mean reaction time both were continuous variables, a regression analysis needed to be done in order to get an adequate view of the results. A p-value of ≤ 0.05 was considered significant.

3.2.8 Medical ethics

The local Medical Ethics Committee of KU Leuven, Belgium approved the protocol (nr: S51615). Furthermore, the protocol was conducted in accordance with the Declaration of Helsinki and its amendments (WMA, 2008).

4 Results

4.1 Primary outcome measures

4.1.1 GABA-concentration in M1 and occipital cortex

When the primary outcome measures were considered, it was important to include the GABA-concentration in M1 (fig 8). There is no significant difference between younger (<30 years of age) and older (<74 years of age) adults. The mean scores $\pm$ SD with the p-values are going to be reported. The outcome of the non-parametric Wilcoxon Signed Rank-test pointed out a probability of .0966, with 2.667 $\pm$ 0.236 ppm measured in the younger adults group and 2.606 $\pm$ 0.345 ppm measured in the older adults group.
Furthermore, when the GABA-concentration in the occipital cortex was considered, there was no significant difference between younger and older subjects either, the non-parametric Wilcoxon Signed Rank-test pointed out a probability of .6054, with a mean of 2.778 $\pm$ 1.138 ppm measured in the younger adults group, and 2.868 $\pm$ 2.951 ppm measured in the older adults group. Outliers were not corrected for in both measurements.

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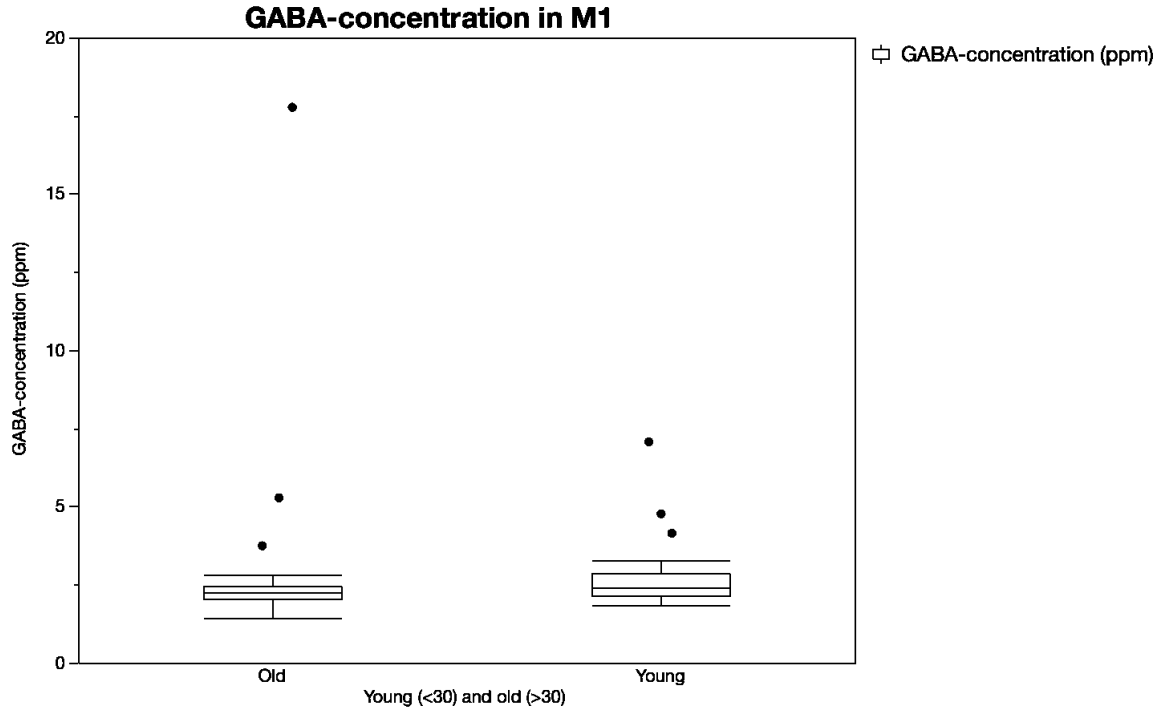

Fig 8: graphical illustration of the mean GABA-concentration in younger and older adults

4.2 Secondary outcome measures

4.2.1 Mean reaction time in M1 and the occipital cortex

Considering the secondary outcome measures, it was important to include outcome measures relating to the go/no go task. The first parameter in this section is the mean reaction time in M1 (fig 9). The mean scores ± SD with p-values are going to be reported. If the comparison was made between younger (<30 years of age) and older subjects (<74 years of age), no significant difference was found in terms of mean reaction time. The probability of the Wilcoxon Signed-Ranked test was .4638, with a mean of 840.416 ± 55.285 ms measured in the younger adults group, and 823.855 ± 40.534 ms measured in the older adults group. At second hand the same comparison was done in the occipital cortex. When the mean reaction time was compared between younger and older adults there was no significant difference as a result of the Wilcoxon signed-Rank test, with a probability of .4124, with a mean of 839.724 ± 54.231 ms measured in the younger adults group, and 823.881 ± 41.277 ms measured in the older adults group. Outliers were not corrected for in both measurements.

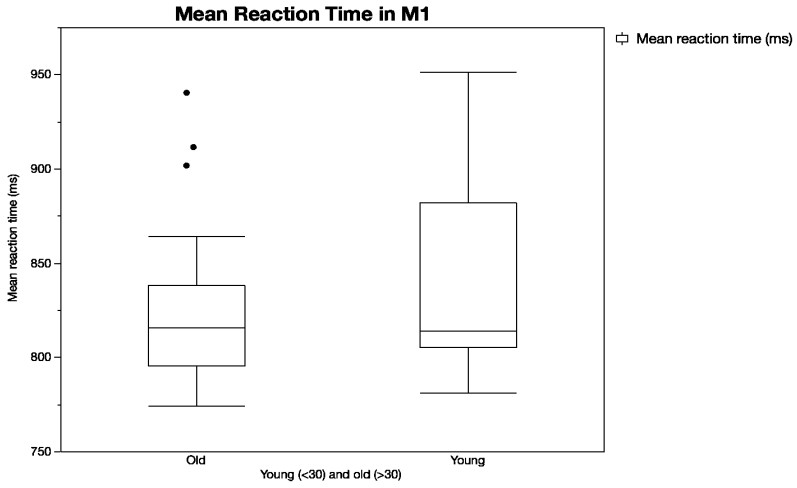

Fig 9: graphical illustration of the mean reaction time in younger and older adults in M1.

4.2.2 SSRT in M1 and the occipital cortex

Moreover, resembling comparisons were made in terms of SSRT. When the parameter was compared between younger and older subjects in M1 (fig 10), a significant difference was found with a probability of .0004, with a mean of 231.910 $\pm$ 22.410 ms measured in the younger adults group, and 251.888 $\pm$ 15.617 ms measured in the older adults group. As follows, SSRT was compared between younger and older subjects in the occipital cortex. When the parameter was compared a significant difference between younger and older subjects was found as estimated by the Wilcoxon Signed-Rank test. The test pointed out a probability of .0002, with a mean of 232.107 $\pm$ 21.961 ms measured in the younger adults group, and 252.425 $\pm$ 15.628 ms measured in the older adults group. Outliers were not corrected for in both measurements.

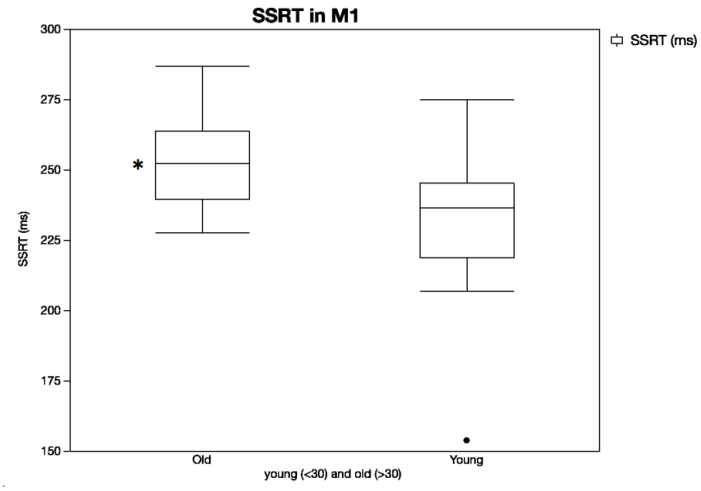

Fig 10: graphical illustration of SSRT in younger and older adults. *indicates a significantly higher SSRT; Wilcoxon Signed-Rank test.

4.2.3 Correlations between SSRT, GABA-concentration and mean reaction time in M1

In addition to this, the investigation of any correlations between SSRT and mean reaction time at one hand and GABA-concentration at the other hand were important outcomes considering the hypothesis. SSRT, mean reaction time and GABA-concentration are all continuous variables which is why a multiple linear regression statistic method was used to correlate these variables. Because normality and/or validity assumptions concerning the residuals of all secondary outcome measures were not met, the choice was made to conduct a non-parametric regression model of all secondary outcome measures. As pointed out by the non-parametric regression model by using a Spearman's Rank-Order correlation no significant relationship was found between mean reaction time and the GABA-concentration in M1 (p=.2197) (r=.1714) (fig 11A). Furthermore, when the correlation between SSRT and GABA-concentration was estimated no significant result was found either (p=.4067) (r=-.1164). In addition to this neither did the correlation of SSRT and mean reaction time estimate any significant results (p=.1831) (r=-.1857) (fig 11B). Outliers were not corrected for in both measurements.

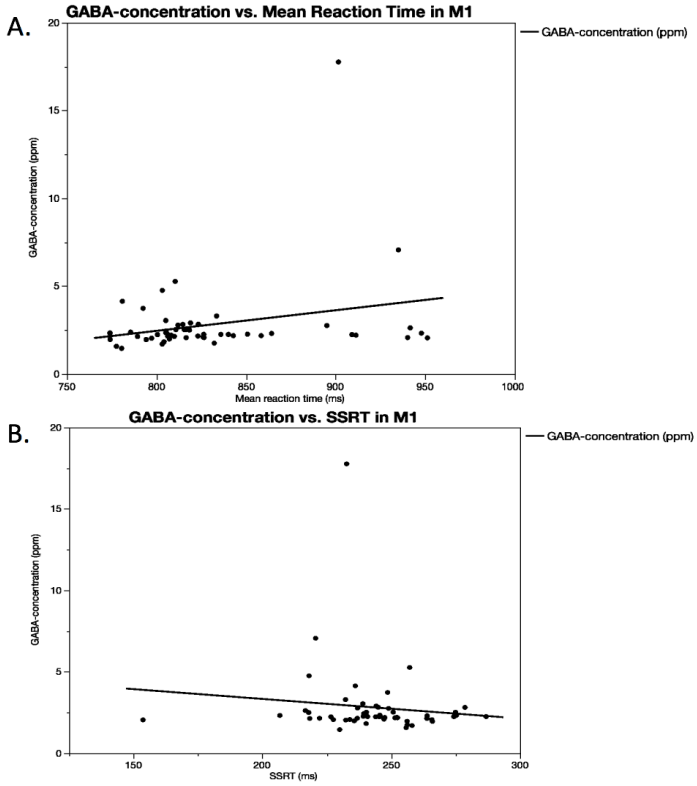

Fig 11 A/B: graphical illustration of the correlation between the GABA-concentration and mean reaction time (A) and the GABA-concentration and SSRT (B).

4.2.4 Correlations between SSRT, GABA-concentration and mean reaction time in the occipital cortex

Furthermore, the same algorithm was executed for the results in the occipital cortex. Because the assumptions of normality and/or the validity in all secondary outcome measures were not met, the choice was made to do a non-parametric regression analysis. As pointed out by this non-parametric regression analysis by using a Spearman's Rank-Order correlation the result between mean reaction time and GABA-concentration was not significant (p=.4773) (r=-.0997). Moreover, when the correlation was estimated between SSRT and the GABA-concentration, the result was not significant either (p=.3524) (r=- .1303). Definitively, neither was the correlation between SSRT and mean reaction time significant (p=.1831) (r=-.1857).

4.2.5 Synopsis

Conclusively, no significant results were found in the primary outcome measures. This concretely means that there was no difference in GABA-concentration between younger and older adults, concerning M1 and the occipital cortex. In the secondary outcome measures, only one variable was found significant. SSRT was significantly higher in older adults in comparison to their younger counterparts in the occipital cortex and M1. In addition to this, mean reaction time did not show any significant difference between younger and older adults, M1 and the occipital cortex considered. Moreover, no significant relationship was found between mean reaction time, SSRT and GABA-concentration. These, non-significant, negative relationships were found in M1 and in the occipital cortex, with the exception of one, non-significant, positive correlation between the GABA-concentration and the mean reaction time in M1 and in the occipital cortex. This particularly summarizes that although there was a non-significant correlation between mean reaction and GABA-concentration and between SSRT and GABA-concentration, a tendency of an increased mean reaction time and a decreased SSRT was found with increasing GABA-concentrations, irrespectively of the subject's age.

5 Discussion

The current study aimed to investigate the difference between the GABA-concentration in younger and older adults and intended to examine any difference between mean reaction time and stop signal reaction time (SSRT) in both age groups. Noteworthy, only SSRT was significantly higher in older adults in M1. Remarkably, no significant difference in GABA-concentration was found between age groups. Additionally, correlations between SSRT, mean reaction time and GABA-concentration were conducted and compared between age groups. Moreover, relationships of SSRT and mean reaction time with GABA-concentration were examined, no significant relationship between the different variables was investigated. This is one of the few studies correlating SSRT and mean reaction time to GABA-concentration.

5.1 GABA-concentration in M1 considering different age groups

Noteworthy, no significant difference between age groups was found considering the GABA-concentration in M1 in the specific study population, whereas Gaetz et al., 2011 pointed out that with an increase in age, the GABA levels decreased. Nevertheless, the GABA measures were obtained from a single voxel, which is a limitation of the study and an important difference in the protocol compared to this work, where two voxels were used. One for the occipital cortex and one for M1 (cfr. Method), this could explain why differences are investigated (Gaetz, Edgar, Wang, & Roberts, 2011). Furthermore, Grachev and Apkarian, 2001. did also report a decrease in GABA with an increasing age in the dorsolateral prefrontal cortex (Grachev & Apkarian, 2001). In addition to this Gao et al., 2013 endorses Gaetz et al., 2011 and presented a negative correlation between age and GABA-concentration found in M1, indicating that with an increase in age, the GABA-concentration decreases. GABAergic neuronal loss with increasing age seems to be the cause of this reported result (Gao et al., 2013). However, no decline in grey matter tissue fraction was observed in older adults, indicating that there is no overall decline in total neuron numbers. As a matter of fact the ratio of GABAergic neurons in comparison to the total neural population declines with increasing age (Hua, Kao, Sun, Li, & Zhou, 2008). Although, Grachev et al., 2001 reports that the decline in GABA-concentration is influenced by a decrease in the

number of neurons and synaptic connections in the prefrontal cortex, concerning non-pathological aging. In addition to this, the amount of Cerebrospinal Fluid (CSF) can increase as a result of the grey –and white matter loss, experienced by aging. In respect to this knowledge, more loss of both brain matters, increases the CSF volume apprehended by the voxels, which clouds the possibility of an accurate GABA-concentration assessment in the specific brain region. Anyhow, this effect only seems to be significant in subjects older than the age of 60 (Grachev & Apkarian, 2001). This hypothesis however, is contradicted by Porges et al., 2017. They state that the decline in GABA-concentration in relation to aging is not affected by CSF voxel fraction (Porges et al., 2017). Bargon et al., 1995 also reports neuronal loss as a result of non-pathological aging, described as the reduction of cortical neurons during a human life period (Bargon, Sanders, Caspary, & Buhl, 1995; Pakkenberg & Gundersen, 1997; Terry, DeTeresa, & Hansen, 1987). Interestingly, the leftover synaptic connections become stronger to compensate for the decline in neurotransmitter concentration to preserve an optimal brain function in and across brain regions. This results in more synaptic connections in which the dendrites of the existing neurons will lengthen, to adapt for neuronal loss (Flood, 1993; Flood & Coleman, 1993). Thus, neural mechanisms compensate for the neuronal loss experienced. Another possible explanation for the GABA decline is the relationship between genes coding for glutamic acid decarboxylase (GAD), GAD is a transaminase involved in the production of the neurotransmitter GABA. Hence, it is possible that a dysfunction in the transaminase with an age related decline, contributes to an age related reduction in GABA-concentration (Caspary, Milbrandt, & Helfert, 1995; Leventhal, Wang, Pu, Zhou, & Ma, 2003; Raza, Milbrandt, Arneric, & Caspary, 1994). Taken into consideration the process of aging, cognitive decline can be a contributor to the decline in GABAergic inhibition (Caspary et al., 1995; Yang et al., 2009; Zhang et al., 2008). Furthermore, Gao et al., 2013 reported the use of Creatine (Cr) to express GABA+ levels, where contradictions in literature considering age-related Cr changes are apparent. Some studies show no significant age-related change (Brooks et al., 2001; Christiansen et al., 1993), other show an age-related increase in Cr (Bargon et al., 1995; Maudsley et al., 2009; Reyngoudt et al., 2012; Robertson et al., 2001; Saunders et al., 1999). Hence, the possibility that the age related change in GABA-concentration described in the work of Gao et al., 2013, is influenced by an age-related increase in Cr. Moreover, gender differences can be an important contributor in GABA-concentration changes. Gao et al., 2013 reported that no

gender specific difference was found considering GABA-concentration in the frontal and parietal regions of the brain (Gao et al., 2013). Although, it has previously been reported that the menstrual cycle can influence the GABAa receptor expression (Lovick, 2006). Whereas, other studies concluded that GABA levels were significantly higher in men than women in the dorsolateral prefrontal cortex (O'Gorman, Michels, Edden, Murdoch, & Martin, 2011). Gao et al., 2013 reported that in their research protocol no influence of the contraceptive status of the female subjects were recorded (Gao et al., 2013), this could elucidate the difference between their outcome and other studies, including this work. In addition to this, it has been previously reported that the frontal region of the brain, including M1, is affected by aging (Brooks et al., 2001). Moreover, the edited GABA-signal detected had a significant contribution of co-edited macromolecules and Homocarnosine. Although the in vivo concentration of GABA is much higher than the in vivo concentration of Homocarnosine (Govindaraju et al., 2000). This explains the unlikeliness that changes in Homocarnosine could influence the GABA-concentration (Gao et al., 2013).

5.2 Mean reaction time in M1 considering different age groups

In the current work no significant correlation was found between GABA-concentration and mean reaction time, although a tendency for an increase in mean reaction time was found, with an increasing GABA-concentration. In addition to this, no significant difference between age groups was examined considering mean reaction time. Stagg et al., 2011 reported a significant positive correlation between mean reaction time and GABA-concentration in favor of younger adults (Stagg, Bachtiar, et al., 2011). One could speculate that a larger population sample in this work could have led to a significant correlation between GABA-concentration and mean reaction time. Moreover, the comparison between older and younger adults in terms of reaction time, pointed out a significant difference, where older adults experience longer reaction times in comparison to their younger counterparts as reported by Cuypers et al., 2013. Age related increase in reaction time is well reported in recent studies (Jordan & Rabbitt, 1977). Age related increase in mean reaction time happens, due to a stronger and longer activation need in contralateral motor cortex, to trigger a motor response (Falkenstein, Yordanova, & Kolev, 2006; Yordanova, Kolev, Hohnsbein, & Falkenstein, 2004). However, it is unclear that the observations reflect deficits

in response-generation, in movement preparation, or in both (Cuypers et al., 2013). Cuypers et al., 2013 did also observe a difference for MEP suppression in the preparatory period to a reaction task, younger adults have a significantly better MEP suppression in the dominant finger in comparison to their older counterparts. Less suppression in MEP is correlated with slower reaction times, as seen in older adults. However, older adults can compensate for a deficient motor activation. Older adults have the capability to increase their corticospinal excitability and thus decrease their MEP suppression prior to the signal of a simple reaction task (Cuypers et al., 2013). Older adults show increased readiness to the upcoming go signal by increasing corticospinal projections to the active hand while they reduce the descending tract projections of the hand at rest (Levin et al., 2011). Anyhow, these findings are discussed to be mechanisms that help older adults to be more efficient in conducting a voluntary motor response (Sinclair & Hammond, 2009). In respect to this knowledge, one can speculate that caution is a measure needed to be taken in interpreting results considering reaction time in older adults between 65 and 75 years of age, when a simple reaction task is executed. The most justifiable interpretation of these findings is that the decline in preparatory process in the dominant hemisphere is directly linked with slower reaction times measured in older adults. Evidence from previous studies pointed out that RT decreases in short (500– 1.000 ms) preparatory periods prior to a reaction task, are accompanied by reduction in corticospinal excitability at the expected onset of the stimulus (Davranche et al., 2007; Duque & Ivry, 2009; Duque, Lew, Mazzocchio, Olivier, & Ivry, 2010; Fujiyama et al., 2011; Hasbroucq, Kaneko, Akamatsu, & Possamai, 1997; Hasbroucq et al., 1999; Sinclair & Hammond, 2008, 2009; Tandonnet, Burle, Vidal, & Hasbroucq, 2003; Tandonnet, Garry, & Summers, 2010). Nonetheless, when the time of EMG onset is taken into consideration, a significant decrease in intracortical inhibition and increase in corticospinal excitability was found in younger adults when the go response was signaled. The older counterparts showed a comparable reduction in intracortical inhibition, as the corticospinal excitability knew a significantly smaller increase at EMG onset, when compared with younger adults (Fujiyama et al., 2011), suggesting that older adults have slower reaction times in comparison to their younger equivalents. However, the event regarding suppression of MEPs in combination with an increased reaction time in older adults seems contradictory, regardless, several studies examined that an increased activation of inhibitory interneurons is correlated in a negative manner with reaction time (Cuypers et al., 2013). In

respect to the above mentioned findings one could hypothesize, that a better inhibitory function is related to faster reaction times in older adults. Fujiyama et al., 2011 endorses this hypothesis by their recent findings. They reported that an increased regulation of inhibitory capacity was correlated with better performance outcomes in older adults (Fujiyama et al., 2011). Nonetheless, it can not be neglected that subcortical and/or spinal levels of the motor system are associated with MEPs and thus can influence motor performance (Duque & Ivry, 2009; Duque et al., 2010; Fujiyama et al., 2011; Sinclair & Hammond, 2008, 2009; Tandonnet et al., 2003; Tandonnet et al., 2010). Yet, in the onset to the voluntary motor response no significant difference was measured in MEP facilitation between both age groups (Cuypers et al., 2013). Conclusively, as age increases motor slowing is experienced, this can be influenced by a depletion of testosterone and increasing cortisol levels in combination with attention deficits seen in elderly (Fontani, Lodi, Felici, Corradeschi, & Lupo, 2004). Testosterone has got a direct effect on neural excitability. Therefore, a lower testosterone level can have an effect on MEP levels in comparison to younger equivalents (Reddy, 2009), declaring the slower reaction times experienced in older adults.

5.3 Contribution of SICI and LICI in mean reaction time, measured in M1

SICI represents GABAa inhibitory activity. When the GABAa mediated inhibitory activity is taken into consideration, Heise et al., 2013 found a decrease in SICI corresponding with an increasing age in resting conditions. The level of resting state inhibition modulated the event-related release of inhibition. This supports the hypothesis that motor performance is dependent on GABAa mediated inhibition. Because SICI and age are correlated to each other, and event-related release of inhibition is dependent on GABAa mediated inhibition, one could hypothesize that age also has an influence on event related inhibition release, however this is not investigated properly and no clear interpretation could be made at this point in time (Heise et al., 2013). Furthermore, stronger resting-state inhibition is correlated with better motor performance, it is considered that GABAa mediated inhibition has a fine-tuning effect for neural firing. Because a decrease in SICI is found with increasing age, more disinhibition takes place. When the disinhibition becomes more prevailing it might negatively impact the precision of the execution and timing of the motor task. Knowing this it can be hypothesized that the mean reaction time could increase. Because, motor

processing is more impaired in case of disinhibition in older adults, the response generation will be slowed (Heise et al., 2013). However, in more complex manual motor tasks, such as alternating two-finger tapping, stronger event-related inhibition modulation was found, which is a logical finding, hence with an increase in event-related inhibition the association with an increase in resting state inhibition can be made. This is associated with more GABAa activity and a better performance. Moreover, concerning reaction time, it is important to consider the cognitive load. When the cognitive load of a motor task is sizable, the motor processing is more complex. The hypothesis could be made that event-related modulation of inhibition estimates the efficiency of the inhibitory system in synaptic integration. This inhibitory efficiency is reduced significantly when the GABAa activity is weakened as seen in older adults (Heise et al., 2013). One could hypothesize that mean reaction time changes as a result of the reduction in inhibitory efficiency. Furthermore, as pointed out by Opie and Semmler., 2014 SICI in active muscle and LICI in resting muscle were decreased in older adults in comparison to their younger counterparts. These differences were not found with SICI in resting muscle and LICI in active muscle muscle (Opie & Semmler, 2014). In this particular example it could be hypothesized that only SICI has an influence on the efficiency of the motor task preformed and in that manner also influences reaction time. This hypothesis is supported by Heise et al., 2013 as mentioned in the paragraph above. Although, cautious approach in interpreting these results must be considered, as the age related difference in SICI was dependent on MEP amplitude (Opie & Semmler, 2014). Opie et al., 2013 point out that LICI in active muscle at low test TMS intensities and test MEP amplitudes (<2mV) is elevated by increasing age. When the MEP amplitudes normalized the difference diminished. The contradictory results could however be declared by the different inter stimulus intervals between the two pulses generated using a LICI protocol. Anyhow, the results show that LICI may be reduced in older adults, as is seen in SICI (Opie & Semmler, 2014). One could hypothesize that these two inhibitory processes are more related to each other than previously investigated. In addition to this, contraction intensities can have an important influence on LICI. Interestingly, with low TMS intensities and low MEP amplitudes, as mentioned above, younger subjects demonstrated MEP facilitation, whereas older subjects demonstrated MEP inhibition (Opie & Semmler, 2014), suggesting that older subjects could have longer reaction times in comparison to their younger counterparts.

5.4 SSRT in M1 considering different age groups

As represented by the current work a significant difference between younger and older adults was found concerning SSRT. Older adults experienced significantly higher SSRTs in comparison to their younger equivalents. However, in the contradictory, Van den Wildenberg & Van der Molen., 2004 found that with an age increase, the SSRT became shorter (van den Wildenberg & van der Molen, 2004). This difference however can be explained by the discrepancy considering the age groups used in both study populations. Williams et al., 1999 endorses the findings of this work and stated that the ability to inhibit responses enhances during childhood and diminishes when growing into adulthood. Resemblance between both age groups point out that older adults, indeed, have longer SSRTs in comparison to their younger counterparts, however older adults were only 20 ms slower (Williams, Ponesse, Schachar, Logan, & Tannock, 1999). It supports the findings as presented in this work. Although, Kramer et al., 1996 investigated a more profound slowing in SSRT compared to the younger equivalents, older adults were 90 ms slower in comparison to the previous 20 ms reported in Williams et al., 1999. However, a more complex go task was used by Kramer et al., 1996, this could contribute to the observed difference, because a more complex go task is related to a greater difficulty in controlling the stop-process (Kramer, Humphrey, Larish, Logan, & Strayer, 1994). Nonetheless, caution is a mandatory measure in the interpretation of these results, because age is not the only factor contributing to SSRT. Individual differences in inhibitory capacity also play a key role in relation to these outcome measures (Williams et al., 1999). In this respect, personality traits may also be contributors. Impulsivity traits may be related to a decrease in inhibitory capacity and could have an influence on the reported results in SSRT. Furthermore, impulsivity seems to be related to inhibitory processes only, not to response execution processes (Gordon D. Logan, 1997). Another endorsement of the findings in this work was assessed by Kramer et al., 1994. The results pointed out that older adults exhibit a longer SSRT in comparison to their younger counterparts (Kramer et al., 1994). Older adults are considered to take longer to implement inhibitory processes, in comparison to their younger equivalents. Interestingly, the longer SSRT could be influenced by general slowing (Kramer et al., 1994).

5.5 Correlations between SSRT and/or GABA-concentration and/or mean reaction time in M1

There is a decrease in mean reaction time and an increase in the latency of the reaction to the stop signal (SSRT) with a decreased GABA-concentration in elderly as compared to their younger counterparts. Verbruggen et al., 2009 supports the hypothesis that SSRT and RT are two different measures. They state that when the comparison of go RT and SSRT is considered, it seems that go and stop performance develop and decline independently of each other (Verbruggen & Logan, 2009).

5.6 Strengths and limitations

The results of this study should be interpreted carefully. First of all, because of the relatively small sample size (n=53), the power of the current study is rather small and therefore the representability reduces. Moreover, the MRS-measures and go/no go task were executed at separate days, possibly influencing the GABA-concentration and therefore affecting the correlation between the behavioral measures and the GABA-concentration in vivo. Additionally, the measure of total GABA-concentration within a specific voxel does not give information concerning the subcellular localization of GABA and thus the measurement of the concentration remains speculative (Stagg et al., 2009). Moreover, the usage of smaller voxels may specify the target brain regions in which the GABA-concentration can be measured (Gao et al., 2013). Most of the GABA-spectra obtained by the voxels are a mixture of grey matter, white matter and CSF. More accurate measures considering the spectra could be obtained when each segment (white matter and gray matter), was investigated individually (Grachev & Apkarian, 2001). However, a big strength of this study was the standardization of the protocol used at each day of measurements and the thorough screening for possible comorbidities and influencing factors. Another strength of this study is the explicit mention of the in–and exclusion criteria and the comparable amount of subjects in both age groups. Conclusively and most importantly this is one of the few studies to correlate GABA-concentration, mean reaction time and SSRT in both younger and older adults.

5.7 Recommendations for future research

First and foremost, more thorough examination of other macromolecules influencing the GABA-concentrations in MRS measurements is needed, to interprete results more accurately. Moreover, more differentiation between white and grey matter GABA-concentrations has to be made, despite the extended methodological procedure needed to do so. Because GABA-concentration is an important contributing factor in motor execution, more research investigating GABA-concentration, SSRT and mean reaction time and the correlations between these variables in the different age groups should be conducted. Furthermore, at this point in time it is unclear to which extent other brain areas are involved in the execution of motor tasks. This in particular is an interesting subject to investigate. Obtaining more knowledge considering the physiological mechanisms underlying motor execution, and the influence of the different brain areas, can aid in the understanding of age related changes in the execution of motor tasks. Furthermore, many of the studies include smaller sample sizes decreasing the statistical power of any given result. Therefore, it is indicated in future studies to conduct experiments with larger sample sizes. Conclusively, many mechanisms (spinal excitability, intracortical inhibition, aging, gender, other macromolecules, etc..) contribute to GABA-concentration and motor execution for that matter. In respect to this knowledge all these mechanisms need to be investigated separately to acquire the best possible interpretation of these results and results in future studies.

6 Conclusion

In contrast to the hypothesis the results point out that there is no significant difference in GABA-concentration when younger adults are compared to their older equivalents. In addition to this, no significant differences between both age-groups were found concerning mean reaction time. However, a tendency of a positive correlation between GABA and mean reaction time was found, hypothesizing that younger adults experience faster reaction times in comparison to their older counterparts. Nonetheless, these results were non-significant and need to be interpreted carefully. Ultimately, a significant difference between age-groups was measured considering SSRT, where older adults exhibit a longer SSRT in comparison to their younger counterparts. SSRT and GABA show a tendency of an inverse relationship to each other. However, all the aforementioned results should be interpreted with caution because small sample sizes were used, thus reducing representability. Ultimately, more research is recommended to fully understand the process of motor inhibition.

7 References

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8 Appendices

8.1 Appendix 1: Edinburgh Handedness Inventory

Edinburgh Handedness Inventory

Surname_____Given Name_____

Date of
Birth_____Sex_____

Please indicate your preferences in the use of hands in the following activities by *putting + in the appropriate column*. Where the preference is so strong that you would never try to use the other hand unless absolutely forces to, *put ++*. If any case you are really indifferent put + in both columns.

Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
i. Which foot do you prefer to kick with?		
ii. Which eye do you use when using only one?		

L.Q.	Leave the spaces blank	DECLE

8.2 Appendix 2: Screening and training protocol young

Screening protocol

Meet participant in the lobby.
Go to 00.19
Fill out/check following forms
- Ask if they understood the 'participant's information sheets'. Go through the forms with the participant.
- Fill out:
  - ☐ Informed consent form
  - ☐ Beck's questionnaire
  - ☐ MRI screening (no metal?)
  - ☐ Medical questionnaire (healthy?)
  - ☐ Oldfield handedness (RH ≥ 70?)
    See form for instructions
  - ☐ F1 form (*payment, for students: fill out bank account number at KU Loket and sign sheet!*)
  - ☐ Baecke's questionnaire
    See form for instructions.

Training Protocol

'U krijgt zometeen lijnen op het scherm te zien met aan het begin van de lijn een bolletje. Als het bolletje begint te bewegen dient u deze te volgen met de cursor op het scherm. Deze cursor kunt u bedienen door gebruik te maken van deze roterende schijfjes. U pakt de pinnetjes vast tussen wijsvinger en duim en door roterende bewegingen beweegt de cursor. Het linker schijfje controleert de beweging op de verticale as en het rechter schijfje op de beweging op de horizontale as. Als u het linker schijfje naar links roteert, beweegt de cursor naar beneden, en naar rechts beweegt de cursor naar boven. Roteert u het rechter schijfje naar links beweegt de cursor naar links, roteert u naar rechts, dan beweegt de cursor naar rechts. Schuine bewegingen maakt u door beide schijfjes tegelijk te roteren. Het doel is om met uw cursor zo dicht mogelijk bij het bolletje te blijven.'

Bimanual control:

Open BTT10_PVR

Practice2 (1x 8 runs for younger participants):

- Enter- participant initials *tab* 'Training' in 'Subjects.txt'
- Wit pijltje → Play
- Select: Participant initials
- Select: CRUNCH_training1
- Select: Run1, Run2, Run3, Run4, Run5, Run6, Run7, Run8
  - Determine score of practice8
    - In Matlab run Analyse.m, change root folder in script, run, open result.txt in Excel, calculate average accuracy per trial type)

      - 1:1 average accuracy > 67%
      - 1:2 average accuracy > 65%
      - Angle average accuracy > 63%
      - Zig-zag average accuracy > 55%

Practice once

Purdue Pegboard *(3x30 sec)*

'Bedoeling van de eerste twee subtests is zo veel mogelijk pinnetjes met de desbetreffende hand in het bord te steken binnen 30 seconden. Bij de derde subtest gaat het om met beide handen zoveel mogelijk paren pinnetjes in het bord steken. Deze 3 subtests zullen in deze studie uitgevoerd worden.

Per subtest wordt als volgt gescoord:
1. Het aantal pinnetjes op het bord.
2. Het aantal pinnetjes op het bord.
3. Het aantal paren pinnetjes op het bord.

Als pp een pinnetje laat vallen dan moet hij dit laten liggen, een nieuw pinnetje nemen en verder doen. Wanneer ze linker en rechter hand samen moeten doen, moeten ze beide pinnetjes ook samen in de gaatjes steken (en dus niet één per één!)

Stop-signal task *(i.e. Inhibition – 8min)*

Go to: Documents\CRUNCH\Training\Inhibition\Inhibition_training
Start 'Inhibition'
'*Load slide settings'* → *'CRUNCH'*
'Load trial settings' → '*CRUNCH'*
'Load run' → '*CRUNCH_training_xxxx*

Instructies
'Plaats uw rechter hand op het vlak. Zo meteen zie je een balk zich van onder naar boven vullen met een blauwe kleur. Op het moment dat de kleur de horizontale streepje bereikt moet u uw hand zo snel mogelijk van het vlak afhalen en terugplaatsen. De horizontale streepjes worden groen, oranje of rood. Groen betekent: goed gedaan. Oranje betekent: ok, maar het kan beter. Rood betekent: het moet beter.
Soms stopt het vullen van de balk plots. U moet dan uw hand op het vlak laten liggen, dus niet optillen. Succes'

Multi Limb Reaction Time task *(8 min)*

Instructies
'Voor deze test dient u uw schoenen uit te doen. Plaats uw handen en voeten op de pads. Zo meteen ziet u vier vlakken. Deze vlakken corresponderen met uw handen en voeten, zo representeert het vlak links boven uw linker hand, en het vlak rechtsonder uw rechter voet. Deze vlakken kunnen blauw worden waarop u zo snel mogelijk de desbetreffende hand/voet moet optillen en rustig terug moet plaatsen. Het kan zijn dat er meerdere balken blauw worden. U dient dan ook meeredere ledematen zo snel mogelijk op te tillen. Is dit duidelijk?'

Place hands and feet on pads.
Make sure the participant is comfortable and is able to lift hands and feet quickly. Adjust position if needed..

Start *'MuLRT_CRT'* (It will run itself)

Local/Global task (8 min)

'Zo meteen ziet u telkens een vierkant of een rechthoek in beeld verschijnen die op hun beurt zijn opgebouwd uit kleine vierkantjes of rechthoeken. Voordat deze figuren verschijnen ziet u eerst een aanwijzing die aangeeft waarop u moet letten, ofwel de kleine figuren ofwel de grote figuren. Is de figuur die u ziet een vierkant dan drukt u zo snel mogelijk op '1', is het een rechthoek, dan drukt u zo snel mogelijk op '2'.'

'De taak zal nu nog eens worden uitgelegd'

Start *'LocalGlobal'*
(Subject: 'subj nr., Session 9. Naam: 'initialen' – 10 min.)

8.3 Appendix 3: screening and training protocol old

Screening protocol

Meet participant at the lobby.
Go to 00.19
Fill out/check following forms
- Ask if they understood the 'participant's information sheets'. Go through the forms with the participant.
- Fill out:

- ☐ Informed consent form
- ☐ Beck's questionnaire
- ☐ MRI screening (no metal?)
- ☐ Medical questionnaire (healthy?)
- ☐ Oldfield handedness (RH ≥ 70?)
  See form for instructions
- ☐ Montreal Cognitive Assessment (*Fill out from age 60*)
- ☐ F1 form (*payment, for students: fill out bank account number at KU Loket and sign sheet!*)
- ☐ Baecke's questionnaire
  See form for instructions.

Montreal Cognitive Assessment

Valide en betrouwbare test voor (milde) cognitieve beperkingen bij vele onderzoekspopulaties (zie publicaties pubmed)
Test hier te downloaden:
http://www.mocatest.org/pdf_files/MoCA-Test-Dutch.pdf
MoCA Version November 12, 2004 1 © Z. Nasreddine MD, Translated to Dutch by P.L.J. Dautzenberg MD, PhD and J.F.M. de Jonghe PhD www.mocatest.org

Afname- and Scoringinstructies
De Montreal Cognitive Assessment (MoCA) is ontworpen als een beknopt screeningsinstrument voor lichte cognitieve stoornissen. Verschillende cognitieve domeinen worden beoordeeld: aandacht en concentratie, executieve functies, geheugen, taal, visuo-constructieve vaardigheden, conceptueel denken, rekenen en oriëntatie. Afname van de MoCA neemt ongeveer 10 minuten in beslag. Het maximum aantal te behalen punten is 30; een score van 26 punten of hoger wordt beschouwd als normaal.

1. Alternerende Trail Making:
Afname: De onderzoeker instrueert de proefpersoon: "Teken een lijn, van een cijfer naar een letter en in oplopende volgorde. Begin hier [wijs naar (1)] en teken een lijn van 1 naar A, dan naar 2 en zo verder. Stop hier [wijs naar (E)]."
Scoring: 1 punt wordt toegekend indien de proefpersoon het volgende patroon correct tekent: 1-A-2-B-3-C-4-D-5-E, zonder dat de lijnen elkaar kruisen. Een fout die de proefpersoon niet direct zelf verbetert krijgt een score 0.

2. Visuo-constructieve vaardigheden (Kubus):
Afname: De onderzoeker wijst naar de **kubus** en geeft de volgende instructie: “Teken dit figuur zo nauwkeurig mogelijk na, in de ruimte hieronder”.
Scoring: Er wordt 1 punt toegekend voor een correcte tekening.

De tekening moet driedimensionaal zijn
Alle lijnen zijn getekend
Er is geen extra lijn toegevoegd
De lijnen lopen relatief parallel en zijn van gelijke lengte (rechthoekige prisma’s worden geaccepteerd).

Indien aan één van bovenstaande criteria niet wordt voldaan, is de score 0.

3. Visuo-constructieve vaardigheden (Klok):
Afname: Wijs naar de rechter bovenkant van het scoreformulier en geef de volgende instructie: “Teken een **klok**. Plaats er alle cijfers in en zet de wijzers op 10 over 11”.
Scoring: Er wordt één punt toegekend voor elk van de volgende 3 criteria:

Omtrek (1 pt.): de omtrek van de klok moet een cirkel zijn. Hooguit een kleine afwijking is acceptabel (b.v., een kleine onvolkomenheid bij het sluiten van de cirkel);
Cijfers (1 pt.): alle cijfers van de klok zijn aanwezig, zonder toevoeging van extra cijfers; de cijfers staan in de juiste volgorde en moeten ongeveer in de kwadranten van de klok

geplaatst zijn; Romeinse cijfers zijn toegestaan; de cijfers mogen aan de buitenkant van de cirkel geplaatst worden;

Wijzers (1 pt.): er moeten twee wijzers zijn die samen de correcte tijd aangeven; de uurwijzer moet duidelijk korter zijn dan de minutenwijzer; de wijzers moeten in de klok getekend worden en elkaar ongeveer in het midden van de cirkel kruisen.

Er wordt geen punt toegekend voor een element indien aan de bovenstaande criteria niet wordt voldaan.

4. Benoemen:

Afname: Wijs vanaf links ieder figuur aan en zeg: "Hoe heet dit dier?".
Scoring: Voor elk van de volgende antwoorden wordt 1 punt gegeven: (1) leeuw, (2) neushoorn, (3) kameel of dromedaris.

5. Geheugen:

Afname: Onderzoeker leest een rij van 5 woorden voor met een snelheid van één woord per seconde, en geeft hierbij de volgende instructies: "Dit is een geheugentest. Ik ga een rij woorden voorlezen die u moet onthouden, nu maar ook straks. Luister goed. Als ik klaar ben, vertelt u me alle woorden die u hebt onthouden. Het maakt niet uit in welke volgorde u ze opnoemt". Zet een kruisje in de aangegeven ruimte voor ieder woord dat de proefpersoon tijdens deze eerste aanbieding reproduceert. Wanneer de proefpersoon aangeeft dat hij/zij klaar is (alle woorden heeft herinnerd), of zich geen woorden meer weet te herinneren, lees dan de lijst met woorden een tweede keer voor met de volgende instructie: "Ik ga dezelfde lijst een tweede keer voorlezen. Probeer zo veel mogelijk woorden te onthouden en vertel ze me, ook de woorden die u de eerste keer hebt opgenoemd." Zet een vinkje in de aangegeven ruimte voor ieder woord dat de proefpersoon zich herinnert na de tweede aanbieding.

Vertel de proefpersoon aan het einde van de tweede aanbieding dat later nogmaals naar de woorden gevraagd zal worden, door te zeggen: "Ik zal u aan het eind van deze test opnieuw vragen welke woorden u zich nog weet te herinneren."

Scoring: Er worden géén punten gegeven voor aanbiedingen één en twee.

6. Aandacht:

Cijferreeksen vooruit: Afname: Geef de volgende instructie: "Ik ga een aantal cijfers opnoemen en als ik klaar ben, moet u ze in dezelfde volgorde nazeggen als ik ze heb gezegd". Lees de vijf-cijfer reeks met een snelheid van één cijfer per seconde.

Cijferreeksen achteruit: Afname: Geef de volgende instructie: "Nu ga ik weer cijfers opnoemen, maar zodra ik klaar ben, moet u ze in omgekeerde volgorde nazeggen." Lees de drie-cijfer reeks met een snelheid van één cijfer per seconde.

Scoring: Er wordt 1 punt gegeven voor elke correct nagezegde reeks, (N.B.: het correcte antwoord voor cijferreeksen achteruit is 2-4-7).

Volgehouden aandacht:
Afname: De onderzoeker leest de rij letters voor met een snelheid van één letter per seconde. Geef de volgende instructie: "Ik ga u een reeks letters voorlezen. Iedere keer dat ik de letter

A noem, tikt u eenmaal met uw hand op tafel. Wanneer ik een andere letter noem, tikt u niet met uw hand op tafel".
Scoring: Geef 1 punt bij nul of één fout (een fout is een tik bij de verkeerde letter of geen tik bij de letter A).

Seriële 7's:
Afname: De onderzoeker geeft de volgende instructie: "Wilt u van 100 zeven aftrekken en van wat overblijft weer zeven aftrekken en zo doorgaan tot ik stop zeg?" Geef deze instructie zonodig tweemaal.
Scoring: Op dit item zijn maximaal 3 punten te behalen. Geef geen (0) punten indien geen enkele correct is, 1 punt voor één correcte aftreksom, 2 punten voor twee of drie correcte aftreksommen, en 3 punten indien vier of vijf aftreksommen juist zijn gemaakt. Tel iedere juiste aftrekking van 7, beginnend bij 100. Iedere aftreksom wordt individueel beoordeeld; dit houdt in dat, indien een proefpersoon met een foutief getal antwoordt, maar vervolgens correct doorgaat met hier 7 van af te trekken, er een punt voor iedere correcte som wordt gegeven. Een proefpersoon kan bijvoorbeeld antwoorden: "92 – 85 – 78 – 71 – 64" waarbij de "92" fout is, maar alle volgende getallen correct zijn afgetrokken. Dit is één fout en het item krijgt een score van 3.

7. Zinnen nazeggen:
Afname: De onderzoeker geeft de volgende instructies: "Ik ga u een zin voorlezen. Zeg deze na zodra ik klaar ben, precies zoals ik hem heb gezegd [pauze]: **Ik weet alleen dat Jan vandaag geholpen zou worden."** Na het antwoord zegt u: "Nu ga ik u een andere zin voorlezen. Zeg deze na, precies zoals ik hem heb gezegd [pauze]: **De kat verstopte zich altijd onder de bank als er honden in de kamer waren.**"

Scoring: Ken 1 punt toe voor iedere correct herhaalde zin. De herhaling moet precies hetzelfde zijn. Wees alert voor omissies (b.v., "alleen", "altijd" vergeten) en vervangingen/toevoegingen (b.v., "Jan is degene die vandaag heeft geholpen"; "verstopte" vervangen door "verstopt", meervoud veranderen, etc.).

8. Verbale fluency:
Afname: De onderzoeker geeft de volgende instructie: "Noem zo veel mogelijk woorden als u kunt bedenken die beginnen met een bepaalde letter van het alfabet. Ik zal u de letters straks vertellen. U mag ieder woord noemen dat u wilt, behalve namen, cijfers, of woorden die met hetzelfde voorstukje (voorvoegsel) beginnen, zoals bijvoorbeeld lief, liefde, liefdevol. Na één minuut vraag ik u te stoppen. Bent u er klaar voor? [pauze] Noem zo veel mogelijk woorden als u kunt bedenken die beginnen met de letter **D**. [tel 60 sec af]. Stop."
Scoring: Ken 1 punt toe indien de proefpersoon 11 woorden of meer kan opnoemen in 60 seconden. Noteer de antwoorden onderaan het blad, of in de kantlijn.

9. Abstractie:
Afname: De onderzoeker vraagt de proefpersoon uit te leggen wat ieder woordpaar gemeenschappelijk heeft. Begin met het voorbeeld: "Kunt u mij vertellen in welke opzicht een sinaasappel en een banaan aan elkaar gelijk zijn, wat is de overeenkomst tussen beide?". Wanneer de proefpersoon een concreet antwoord geeft, zeg dan slechts één keer extra:

"Weet u nog een andere overeenkomst?". Indien de proefpersoon niet het correcte antwoord geeft (fruit), zeg dan, "Ja, en het is beide fruit." Geef geen extra instructies of verduidelijking. Na de oefenafname, zegt u: "In welk opzicht zijn een trein en een fiets aan elkaar gelijk?". Nadat het antwoord gegevens is, stelt u een tweede vraag: "Vertel me nu in welk opzicht een liniaal en een horloge aan elkaar gelijk zijn". Geef geen extra instructies of aanmoedigingen.

Scoring: Alleen de laatste twee itemparen worden gescoord. Geef 1 punt voor ieder correct beantwoord itempaar. Deze antwoorden worden goedgekeurd:

Trein-fiets = vervoermiddelen, manieren om te reizen, je kunt met beide tochten maken;

Liniaal-horloge = meetinstrumenten, worden gebruikt om te meten.

De volgende antwoorden worden **niet** goedgekeurd: Trein-fiets = zij hebben wielen; Liniaal-horloge = zij hebben cijfers.

10. Uitgestelde recall:

Afname: Onderzoeker geeft de volgende instructie: "Ik heb u eerder een rij met woorden voorgelezen, en ik vroeg u ze te onthouden. Vertel me zo veel mogelijk woorden die u zich kunt herinneren. Zet een vinkje in de daarvoor bestemde ruimte (√) voor ieder correct woord dat de proefpersoon zich spontaan, zonder aanwijzingen, heeft weten te herinneren.

Scoring: Ken 1 punt toe voor ieder woord dat spontaan wordt herinnerd zonder aanwijzingen.

Optioneel:

Na de uitgestelde spontane recall geeft u de proefpersoon voor ieder niet herinnerd woord een geheugensteuntje ('cue') door middel van de semantische cues, die beneden staan aangegeven. Zet een vinkje (√) in het aangegeven gebied als de proefpersoon zich het woord dankzij de categorie- of de meerkeuzecue herinnert. Help de proefpersoon op deze manier met alle niet herinnerde woorden. Indien de proefpersoon zich niet alle woorden weet te herinneren na de categoriecue, laat hem/haar dan kiezen, waarbij u de volgende voorbeeldinstructie aanhoudt: "Welke van de volgende woorden denkt u dat het was, NEUS, GEZICHT, of HAND?"

Gebruik de volgende categorie- en/of meerkeuzecues voor ieder woord, indien van toepassing:

GEZICHT: categoriecue: lichaamsdeel meerkeuze: neus, gezicht, hand
FLUWEEL: categoriecue: soort textiel meerkeuze: spijkerstof, katoen, fluweel
KERK: categoriecue: soort gebouw meerkeuze: kerk, school, ziekenhuis
MADELIEF: categoriecue: soort bloem meerkeuze: roos, madelief, tulp
ROOD: categoriecue: een kleur meerkeuze: rood, blauw, groen
Scoring: **Er worden geen punten toegekend voor woorden die door middel van een cue worden herinnerd.** Een cue wordt alleen gebruikt voor klinische doeleinden en kan de beoordelaar van de test extra informatie geven over het type geheugenstoornis. Bij geheugenstoornissen die veroorzaakt worden door retrievalproblemen, kunnen cues de prestatie verbeteren. Bij geheugenstoornissen die veroorzaakt worden door een falende opslag, verbetert de prestatie niet met een cue.

11. Oriëntatie:

Afname: Onderzoeker geeft de volgende instructie: “Vertel me de datum van vandaag”. Indien de proefpersoon een onvolledig antwoord geeft, moedig hem dan aan door te zeggen: “Vertel me het [jaar, maand, precieze datum, en dag van de week].” Zeg vervolgens: “Vertel nu: hoe heet dit gebouw en in welke stad/plaats zijn we nu?”

Scoring: Geef 1 punt voor ieder correct beantwoord item. De proefpersoon moet de exacte datum en het exacte gebouw noemen (naam van het ziekenhuis, kliniek, kantoor). Er worden geen punten toegekend als de proefpersoon er één dag naast zit wat betreft de dag van de week en de datum (dag van de maand).

TOTALE SCORE: Tel alle subtestscores die aan de rechterkant staan bij elkaar op. Tel er 1 punt bij op voor personen die 12 jaar of minder formele opleiding hebben gehad (gerekend vanaf leeftijd 6 jaar), zodat een maximum van 30 punten mogelijk is. Een uiteindelijke score van 26 of hoger wordt beschouwd als normaal.

De auteurs zijn mevr. M. van Dommele, Dr. PJ. Visser en mevr. drs. K. van de Berg bijzonder erkentelijk voor hun bijdrage aan het vertalen van de Nederlandse versie van de MoCA.

Training Protocol

'U krijgt zometeen lijnen op het scherm te zien met aan het begin van de lijn een bolletje. Als het bolletje begint te bewegen dient u deze te volgen met de cursor op het scherm. Deze cursor kunt u bedienen door gebruik te maken van deze roterende schijfjes. U pakt de pinnetjes vast tussen wijsvinger en duim en door roterende bewegingen beweegt de cursor. Het linker schijfje controleert de beweging op de verticale as en het rechter schijfje op de beweging op de horizontale as. Als u het linker schijfje naar links roteert, beweegt de cursor naar beneden, en naar rechts beweegt de cursor naar boven. Roteert u het rechter schijfje naar links beweegt de cursor naar links, roteert u naar rechts, dan beweegt de cursor naar rechts. Schuine bewegingen maakt u door beide schijfjes tegelijk te roteren. Het doel is om met uw cursor zo dicht mogelijk bij het bolletje te blijven.'

Bimanual control:

Open BTT10_PVR

Practice2 (for older participants):

- Wit pijltje → Play
- Select: Participant initials
- Select: CRUNCH_training2
- Select Practice1, Practice2, Practice3, Practice4, Practice5, Practice6, Practice7, Practice8
  - Determine score of practice8
    - In Matlab run Analyse.m, change root folder in script, run, open result.txt in Excel, calculate average accuracy per trial type)

      - 1:1 average accuracy > 67%
      - 1:2 average accuracy > 65%
      - Angle average accuracy > 63%
      - Zig-zag average accuracy > 55%

Practice once

Purdue Pegboard *(3x30 sec)*

'Bedoeling van de eerste twee subtests is zo veel mogelijk pinnetjes met de desbetreffende hand in het bord te steken binnen 30 seconden. Bij de derde subtest gaat het om met beide handen zoveel mogelijk paren pinnetjes in het bord steken. Deze 3 subtests zullen in deze studie uitgevoerd worden.

Per subtest wordt als volgt gescoord:
1. Het aantal pinnetjes op het bord.
2. Het aantal pinnetjes op het bord.
3. Het aantal paren pinnetjes op het bord.

Als pp een pinnetje laat vallen dan moet hij dit laten liggen, een nieuw pinnetje nemen en verder doen. Wanneer ze linker en rechter hand samen moeten doen, moeten ze beide pinnetjes ook samen in de gaatjes steken (en dus niet één per één!)

Stop-signal task *(i.e. Inhibition – 8min)*

Go to: Documents\CRUNCH\Training\Inhibition\Inhibition_training
Start 'Inhibition'
'*Load slide settings'* → *'CRUNCH'*
'Load trial settings' → '*CRUNCH'*
'Load run' → '*CRUNCH_training_xxxx*

Instructies

'Plaats uw rechter hand op het vlak. Zo meteen zie je een balk zich van onder naar boven vullen met een blauwe kleur. Op het moment dat de kleur de horizontale streepje bereikt moet u uw hand zo snel mogelijk van het vlak afhalen en terugplaatsen. De horizontale streepjes worden groen, oranje of rood. Groen betekent: goed gedaan. Oranje betekent: ok, maar het kan beter. Rood betekent: het moet beter.
Soms stopt het vullen van de balk plots. U moet dan uw hand op het vlak laten liggen, dus niet optillen. Succes'

Multi Limb Reaction Time task *(8 min)*

Instructies
'Voor deze test dient u uw schoenen uit te doen. Plaats uw handen en voeten op de pads. Zo meteen ziet u vier vlakken. Deze vlakken corresponderen met uw handen en voeten, zo representeert het vlak links boven uw linker hand, en het vlak rechtsonder uw rechter voet. Deze vlakken kunnen blauw worden waarop u zo snel mogelijk de desbetreffende hand/voet moet optillen en rustig terug moet plaatsen. Het kan zijn dat er meerdere balken blauw worden. U dient dan ook meeredere ledematen zo snel mogelijk op te tillen. Is dit duidelijk?'

Place hands and feet on pads.
Make sure the participant is comfortable and is able to lift hands and feet quickly. Adjust position if needed.
Start subsequently all variants of '*MuLRT_SRT_x_xx'*

'*Nu doen we nog eens hetzelfde, maar met het verschil dat alle varianten door elkaar verschijnen.*

Start *'MuLRT_CRT'* (It will run itself)

Local/Global task (8 min)

'Zo meteen ziet u telkens een vierkant of een rechthoek in beeld verschijnen die op hun beurt zijn opgebouwd uit kleine vierkantjes of rechthoeken. Voordat deze figuren verschijnen ziet u eerst een aanwijzing die aangeeft waarop u moet letten, ofwel de kleine figuren ofwel de grote figuren. Is de figuur die u ziet een vierkant dan drukt u zo snel mogelijk op '1', is het een rechthoek, dan drukt u zo snel mogelijk op '2'.'

'De taak zal nu nog eens worden uitgelegd'

Start *'LocalGlobal'*
(Subject: 'subj nr., Session 9. Naam: 'initialen' – 10 min.)

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Richting: **master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen**
Jaar: **2017**

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Van Keer-Van Mol, Jo