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Cerebral ageing: Neurophysiological correlates of ageing processes in cortical activation and functional connectivity

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List of abbreviations

Aβ-40	40 amino acids isoform of β-amyloid
Aβ-42	42 amino acids isoform of β-amyloid
ACC	anterior cingulate cortex
AD	Alzheimer's disease
AIC	anterior insula cortex
aMCI	amnestic mild cognitive impairment
ANOVA	analysis of variance
APA	American Psychiatric Association
apoE-gene	apolipoprotein E – gene
apoE4	apolipoprotein E4
APP	amyloid precursor protein
ASL-MRI	arterial spin labeling magnetic resonance imaging
AU	arbitrary unit
BA	Brodmann's area
BASE	Berliner Altersstudie
BDI	Beck`s Depression Inventory
BOLD	Blood-Oxygenation-Level-Dependent
CA++-ion	calcium 2+-ion
CCN	cognitive control network
CERAD	Consortium to Establish a Registry for Alzheimer`s Disease
Ch	channel
CSF	cerebrospinal fluid
CT	computer tomography
dACC	dorsal anterior cingulate cortex
DAN	dorsal attention network
DCM	dynamic causal modeling
DCT	discrete cosine transform
deg	degree
DHT	dihydrotestosterone

DIN A4	German industry norm A4
dlPFC/DLPFC (ldlPFC, rdlPFC)	dorsolateral prefrontal cortex (left and right dorsolateral prefrontal cortex)
DMN	default mode network
dmPFC	dorsal medial prefrontal cortex
DV	dependent variable
E	estradiol
FC	functional connectivity
FDG-PET	fluoro-2-deoxy-D-glucose-positron emission tomography
fMRI	functional magnet resonance imaging
fNIRS	functional near-infrared spectroscopy
HAROLD	hemispheric asymmetry reduction in old age
HHb	deoxygenated hemoglobin
HSP	heat shock proteins
Hz	Hertz
ICA	independent component analysis
ICD	International Statistical Classification of Diseases and related Health Problems
IEG	immediate early genes
IFG (lIFG, rIFG)	inferior frontal gyrus (left and right inferior frontal gyrus)
IFJ	inferior frontal junction
LFO	low-frequency oscillations
LM	Logical Memory
MCI	mild cognitive impairment
MEG	magnetencephalography
MMSE	Mini Mental State Examination
MOCA	Montreal Cognitive Assessment
NFT	neurofibrillary tangles
NMDA	N-Methyl-D-Aspartate
nm	nanometers
O_2Hb	oxygenated hemoglobin
PCA	principal component analysis

PCC	posterior cingulate cortex
PD	Parkinson's disease
PET	positron emission tomography
PFC	prefrontal cortex
PPC	posterior parietal cortex
PPI	psychophysiological interaction analysis
PRNP	prion protein gene
p-tau181	hyper-phosphorylated tau 181
RBD	REM sleep behavior disorder
ROI	region of interest
rsFC	resting state functional connectivity
SAC (lSAC, rSAC)	somatosensory association cortex (left and right somatosensory association cortex)
SD	standard deviation
SN	salience network
SP	senile plaques
SPL	superior parietal lobule
STAC	scaffolding theory of aging and cognition
TMT	trail making test
TREND	Tübinger evaluation of risk factors for early detection of neurodegeneration
T-tau	total tau
TAP	attentional performance test
WHO	World Health Organization
WMS	Wechsler Memory Scale

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Abstract

Age is considered to be the most important risk factor for the development of neurodegenerative diseases. Against the background of an ageing society, research into possible prodromal markers for neurodegeneration is playing an increasingly important role. The detection of cortical activation and functional connectivity of networks at rest and in executive functions is considered to have high predictive power. A better understanding of neurophysiological ageing processes could contribute to the development of new treatment methods.

This dissertation is dedicated to the investigation of age-related changes in cognitive performance, cortical activation, and functional connectivity. Special interest was shown in the brain area of the cognitive control network (CCN). The dissertation includes three studies. In all three studies, functional near-infrared spectroscopy (fNIRS) was used to record cortical brain activity. Besides the long-known method of functional magnetic resonance imaging (fMRI) for measuring cortical brain activity, functional near-infrared spectroscopy (fNIRS) has been established in neurophysiological studies in recent years. fNIRS offers the advantage of valid measurements under realistic conditions, such as social interaction, as the measurement is not performed in a narrow tube as in MRI. Age-related effects on cognitive performance and cortical blood oxygenation were detected using the Trail Making Test (TMT). The Trail Making Test (classically consisting of the subtests TMT-A and TMT-B, in some versions additionally TMT-C) has proven to be particularly suitable for assessing cognitive performance because it tests various neurophysiological parameters such as mental flexibility, working memory, visuomotor processing speed, and executive functions.

The first study aimed at the fundamental verification of the research validity of using block designs in Trail Making Test. Typically, TMT evaluates the time to completion of all tasks. Inter-individual differences in performance are considered through the time to completion. When using a block design with a measurement time of 30 seconds per TMT subtask, for example, the individual performance must be integrated over the number of solved items within this time interval. We investigated the effects of task complexity and working speed on the

hemodynamic response using fNIRS. The subjects completed the TMT (TMT-A and TMT-B) at three different speeds (slow-medium-fast). Two analyses were carried out to illustrate the influences of task complexity and working speed. In addition to the classical measurement of blood oxygenation, a ratio calculation (O_2Hb per solved item) was used to integrate the number of processed items as a performance component. The results confirmed effects for the working speed in the area of the inferior frontal gyrus (IFG) bilaterally. Interestingly, a higher activity of TMT-B compared to TMT-A in the superior parietal lobule (SPL) area was observed only after correction for the number of processed items.

The second study was devoted to the study of age effects on cortical activation in the execution of the TMT. As shown in the previous study, both working speed and task complexity may have effects on cortical oxygen content. Since older people could compensate for cognitive deficits by reducing working speed, this component should be considered by either using a TMT design with three different speed levels or by integrating an additional variable, cortical blood oxygenation per solved item. The latter was applied in the present study. The aim of the study was to investigate the effects of age differences on TMT performance (number of solved items), cortical blood oxygenation (time-controlled; O_2Hb during task performance), and cortical blood oxygenation per solved item (item-controlled; O_2Hb/item). Based on current research, we assumed an age-related slowdown in working speed (fewer solved items) and a compensatory increase in blood oxygenation during TMT. We considered the level of cortical blood oxygenation per solved item to be particularly sensitive with regard to age-dependent performance declines. In accordance with previous research, the results of our behavioral analysis showed a reduction in working speed in old age. At the same time, lower error rates during TMT-A were observed in older subjects, suggesting a shift in the relationship between speed and accuracy in older subjects. At the neurophysiological level, higher O_2Hb values were observed in the older group. Contrary to the time-corrected data, our combined measurement (O_2Hb/item) revealed no higher O_2Hb values per item in older people in the cognitive control network (CCN).

The third study was devoted to the investigation of changes in functional brain organization, which is considered particularly sensitive to age-related restructuring. Particular attention was paid to the differences in functional connectivity between elderly subjects at rest and in task performance. The functional connectivity within the cognitive control network (CCN) and the dorsal attention network (DAN) was recorded using fNIRS. The results demonstrate age influences in the area of the left inferior frontal gyrus (IFG). In addition, a negative correlation between age and functional connectivity was observed in some regions. Contrary to our expectations, no age differences could be observed with respect to the different conditions (resting state vs. TMT).

The results confirm the suspicion of compensation mechanisms and the decrease of cognitive performance with increasing age.

Deutsche Zusammenfassung

Das Alter gilt als der wichtigste Risikofaktor für die Entstehung neurodegenerativer Erkrankungen. Vor dem Hintergrund einer alternden Gesellschaft spielt die Erforschung möglicher Prodromalmarker für Neurodegeneration eine zunehmend wichtige Rolle. Dabei wird der Erfassung kortikaler Aktivierungen sowie funktioneller Konnektivität von Netzwerken in Ruhe und bei Exekutivfunktionen eine hohe Voraussagekraft beigemessen. Ein besseres Verständnis neurophysiologischer Altersprozesse könnte zur Entwicklung neuer Behandlungsmethoden beitragen.

Die vorliegende Dissertation ist der Erforschung altersabhängiger Veränderungen in der kognitiven Leistungsfähigkeit, kortikalen Aktivierung und funktionellen Konnektivität gewidmet. Ein besonderes Interesse bestand im Gehirnareal des kognitiven Kontrollnetzwerkes (CCN). Neben der schon lange in der Medizin etablierten Methode der Magnetresonanztomographie (MRT) zur Messung oberflächlicher kortikaler Hirnaktivität, hat sich in den letzten Jahren die funktionelle Nahinfrarotspektroskopie (fNIRS) in neurophysiologischen Studien etabliert. fNIRS bietet den Vorteil valider Messungen unter realistischen Bedingungen, wie beispielsweise sozialer Interaktion, da die Messung nicht in einer engen Röhre wie beim MRT erfolgt. Die Vorzüge dieser Technologie dienten als Grundlage der vorliegenden Forschung. Zur Beurteilung der kognitiven Leistungsfähigkeit hat sich der Trail Making Test (klassischerweise bestehend aus Teiltest TMT-A und TMT-B, in einigen Versionen zusätzlich TMT-C) in der neuropsychologischen Forschung etabliert, da er verschiedene neurophysiologische Parameter wie mentale Flexibilität, Arbeitsgedächtnis, visuomotorische Verarbeitungsgeschwindigkeit und Exekutivfunktionen prüft. Anhand des TMT wurden altersabhängige Effekte auf die kognitive Leistung und die kortikale Blutoxygenierung überprüft.

Die Dissertation beinhaltet drei Studien.

Die erste Studie hatte die grundlegende Überprüfung der Validität der Verwendung des Trail Making Test in Blockdesigns in der Forschung zum Ziel. Üblicherweise wird beim TMT die Zeit bis zur Fertigstellung der gesamten

Aufgaben gewertet und darüber die interindividuellen Leistungsunterschiede berücksichtigt. Bei Verwendung eines Blockdesigns, mit einer Messdauer von beispielsweise 30 Sekunden pro TMT-Teilaufgabe muss die individuelle Leistung über die Anzahl gelöster Elemente innerhalb dieses Zeitintervalls integriert werden. Wir untersuchten die Effekte der Aufgabenkomplexität und der Arbeitsgeschwindigkeit auf die hämodynamische Antwort mittels fNIRS. Dazu absolvierten die Probanden den TMT (TMT-A und TMT-B) in drei unterschiedlichen Geschwindigkeiten (langsam-mittel-schnell). Zur Darstellung der Einflüsse von Aufgabenkomplexität und Arbeitsgeschwindigkeit wurden zwei Analysen durchgeführt. Neben der klassischen Messung der Blutoxygenierung, wurde über eine Verhältnisrechnung (O_2HB pro gelöstem item) die Zahl der erreichten Einheiten als Leistungskomponente integriert. Die Ergebnisse bestätigten Effekte für die Arbeitsgeschwindigkeit im Bereich des Gyrus frontalis inferior beidseitig. Interessanterweise zeigte sich eine höhere Aktivität beim TMT-B im Vergleich zum TMT-A im Bereich des Lobulus parietalis superior erst durch die Korrektur für die Anzahl gelöster Elemente.

Die zweite Studie widmete sich der Erforschung von Alterseffekten auf die kortikale Aktivierung bei der Ausführung des Trail Making Tests. Wie in der vorherigen Studie dargestellt, können sowohl die Arbeitsgeschwindigkeit als auch die Aufgabenkomplexität Auswirkungen auf den kortikalen Sauerstoffgehalt haben. Da ältere Menschen kognitive Defizite durch eine Reduktion der Arbeitsgeschwindigkeit kompensieren könnten, sollte zu Berücksichtigung dieser Komponente entweder die Verwendung eines TMT-Designs mit drei unterschiedlichen Geschwindigkeitsstufen oder die Integration einer zusätzlichen Variable, der kortikalen Blutoxygenierung pro gelöstem Element erfolgen. Letztere fand in der vorliegenden Studie Anwendung. Ziel der Studie war die Erforschung der Auswirkungen von Altersdifferenzen auf die Leistung beim TMT (Anzahl gelöster Elemente), die kortikale Blutoxygenierung (zeit-kontrolliert; O_2Hb während der Aufgabenerfüllung) und der kortikalen Blutoxygenierung pro gelöstem Element (O_2Hb/Element). Aufgrund der bisherigen Studienlage gingen wir von einer altersbedingten Verlangsamung der Arbeitsgeschwindigkeit (weniger gelöste Elemente) und einer kompensatorisch erhöhten

Blutoxygenierung während des TMT aus. Die Höhe der kortikalen Blutoxygenierung je gelöstem Element hielten wir in Bezug auf altersabhängige Leistungsrückgänge für besonders sensibel. In Übereinstimmung mit früherer Forschung zeigten die Ergebnisse unserer Verhaltensanalyse eine Reduktion der Arbeitsgeschwindigkeit im Alter. Gleichzeitig wurden geringere Fehlerraten während des TMT-A bei älteren Probanden festgestellt, was auf eine Verschiebung des Verhältnisses zwischen Geschwindigkeit und Genauigkeit bei älteren Probanden hindeutet. Auf neurophysiologischer Ebene wurden höhere O_2Hb–Werte in der älteren Gruppe beobachtet. Die Korrektur der Daten für gelöste Elemente (O_2Hb/Element) zeigte im Gegensatz zu den zeitkorrigierten Daten keine höheren O_2Hb-Werte pro Element bei älteren Menschen im kognitiven Kontrollnetzwerk.

Die dritte Studie widmete sich der Untersuchung von Veränderungen in der funktionellen Gehirnorganisation, die als besonders sensitiv für alterungsbedingte Umstrukturierungen gilt. Dabei lag ein besonderes Augenmerk auf den Unterschieden der funktionellen Konnektivität von älteren Probanden in Ruhemessungen und bei Aufgabenausführung. Erfasst wurde die funktionelle Konnektivität im Bereich des kognitiven Kontrollnetzwerkes und des dorsalen Aufmerksamkeitsnetzwerks mittels fNIRS. Die Ergebnisse demonstrieren Alterseinflüsse im Bereich des linken Gyrus frontalis inferior. Darüber hinaus zeigte sich eine negative Korrelation von Alter und funktioneller Konnektivität in einigen Regionen. Entgegen unserer Erwartungen konnten keine Altersunterschiede in Bezug auf die unterschiedlichen Konditionen (Ruhe vs. TMT) festgestellt werden.

Die Ergebnisse erhärten den Verdacht von Kompensationsmechanismen und bestätigen die Abnahme der kognitiven Leistungsfähigkeit mit zunehmendem Alter.

1. General Introduction

1.1. Topic overview

Aging - the organism in constant change. From the day of birth, the organism is subject to a multitude of aging processes. Cell division, maturation, networking, and death. On a histological level, these processes become visible long before they are reflected macroscopically. While the decline in physical capabilities of the musculoskeletal and organ systems has been extensively researched, little is known about cerebral aging. Especially physiological and pathological cerebral aging are often closely related. We are all exposed to the topic of ageing daily, either on our own bodies or by observing the people who are close to us. In addition to the visible physical limitations with increasing age, behavioral changes and reduced cognitive abilities can often be seen. Since humanity has an increasing life expectancy due to improved medical care and increased prosperity, it can be assumed that age-related neurodegenerative diseases will increase in the future. Thus, research into the underlying neuronal processes becomes of enormous importance, especially to enable early detection and therapeutic intervention. Research results of the last decade have surprisingly shown that the brain might be able to compensate for age-related substance loss through functional changes.

With this dissertation, I would like to shed light on the topic of neuropathological aging processes from a neuroscientific perspective. The analysis of changes in cortical activation, connectivity patterns, and detection of endogenous compensation mechanisms offers enormous potential for the development of new and improved therapeutic approaches.

1.2. Cerebral ageing

The demographic development shows a change of the population structure to an ageing society. Global life expectancy rose to over 74.2 years for women and

69.8 years for men due to better medical care, increased prosperity and a decline in the birth rate (World Health Organization, 2018).

The number of people over 65 years of age in Europe is expected to double in the next two decades (Rees et al., 2012). Age also raises the risk of neurodegenerative diseases as for example Parkinson's disease (PD) or Alzheimer's disease (AD). The prevalence of neurodegeneration increases from <1% in 60-year-olds to over 30% in 90-year-olds (Ziegler & Doblhammer, 2009). It is currently estimated that 46 million people worldwide suffer from dementia. According to projections, this number will develop to 152 million in 2050 (Alzheimer's Disease International, 2019).

1.2.1. Physiological ageing

The brain undergoes a physiological change in structure (Fjell & Walhovd, 2010) and function (Hagen et al., 2014) during its entire lifetime. These reorganizations seem to make it more susceptible to the development of neurodegenerative diseases (Fjell et al., 2014). Two models have attempted to classify physiological and pathological aging. The single factor model (continuity hypothesis) explains cognitive decline by a continuous accumulation of physiological change, and dementia as an acceleration of this process affecting all humans. The multiple-factor model, on the other hand, assumes several influences, such as genetics, anatomical conditions and external risk factors, which affect cognition in old age (Beyreuther et al., 2002; Buckner, 2004). The final distinction between physiological and pathological ageing is still unclear. Several endogenous defenses attempt to counteract the aging process continuously and to keep the extent and severity of a disease as low as possible.

With increasing age, all cortical and subcortical tissues are affected by a reduction in volume. According to MRI studies, an annual reduction of 0.5-1% in grey and white matter can be assumed (Courchesne et al., 2000; Pfefferbaum et al., 1994; Walhovd et al., 2005). This is particularly evident in the frontostriatal system, prefrontal cortex (PFC), and hippocampus, where tissue atrophy and white matter changes lead to initial memory problems (Buckner, 2004; Morrison

& Baxter, 2012; Salat et al., 2004). The structural alteration is accompanied by a change in the size and number of synapses. Often, a compensatory increase in synapse size is observed in areas with reduced synapse numbers (de Brabander et al., 1998; Dickstein et al., 2007; Giannakopoulos et al., 1994). Furthermore, modifications of the synaptic endings with protein deposits occur, which impair the function of the glucose and glutamate transporters as well as the Ca++-ion homeostasis (Mattson, 1997, 2000). Synapses play a key role in the structural organization of neurons.

Via cellular heat shock proteins (HSP), the organism has the possibility to regulate degeneration processes. HSPs control the folding of proteins and cell apoptosis. An increase in misfolded protein deposits suggests a malfunction of the HSPs (van Noort, 2008). As another regulating signal molecule, calcium influences cell function, synaptic transmission, and cell death in neurons. Usually, intra- and extracellular calcium concentrations are in equilibrium. In old age, calcium homeostasis is disturbed by a strong intracellular increase in calcium concentration (Buchholz et al., 2007).

Another aspect of physiological aging is the stiffening and calcification of the arteries (S. K. Shankar, 2010). Cerebral tissue atrophy leads to a thickening and entanglement of the blood vessels, which is accompanied by a disturbance of the microcirculation. As a result, the neuronal nerve cells are undersupplied with nutrients and metabolites. The close coupling of the cerebral blood flow and the cellular metabolism consequently leads to a reduction of functionality and number of neurons (S. K. Shankar, 2010).

In addition, age-dependent changes in the gene expression of immediate early genes (IEG), which are essential for the maintenance of neuronal organization and protein synthesis, can be observed. The genes that promote intracellular calcium release are upregulated, whereas those that support synaptogenesis are downregulated (Blalock et al., 2003). The apolipoprotein E - gene (apoE - gene) is associated with cognitive decline, and in particular the apolipoprotein - allele E4 is associated with an increased risk for the development of AD (Bertram & Tanzi, 2008). A protective gene associated with aging processes is the gene for prion protein (PRNP), which reduces oxidative stress (Brown, 1999).

These structural changes have a significant effect on memory performance at the behavioral level. Working memory and episodic memory are particularly affected. Disturbances manifest themselves in memory gaps, reduced working speed, lowered mental flexibility, and perception (Hedden & Yoon, 2006). In contrast, semantic memory as well as the ability to understand the meaning of words remain mostly unaffected by age loss (Denise C. Park et al., 1996; Schaie, 1996). It is discussed whether the brain relies on compensation mechanisms to maintain cognitive abilities (Buckner, 2004). In MRI studies, a more symmetrical cortical activation in old age was observed, a phenomenon that has established itself in science as the HAROLD (hemispheric asymmetry reduction in old age) model. It is assumed that this is a recruitment of additional neural networks to compensate for weakened or atrophied brain areas (Cabeza, 2002).

1.2.2. Pathological ageing

Pathological cerebral aging is associated with diseases such as Parkinson's disease (PD), Huntington’s disease or Alzheimer's disease. At the cellular level, diagnosis is manifested by so-called Lewy bodies in PD, ubiquitinated intra-neuronal inclusions in Huntington’s disease, and senile plaques (SP) and neurofibrillary tangles (NFT) in Alzheimer’s disease (S. K. Shankar, 2010). Dementia is the umbrella term for clinical pictures associated with chronic progressive brain dysfunction, memory loss, cognitive disorders with reduced thinking ability, orientation, learning capacity, language, and emotional control (World Health Organization, 1992). Alzheimer's disease is the most frequently diagnosed variant of dementia. Due to the high prevalence of 46 million people suffering from Alzheimer's disease worldwide, special attention is paid to this clinical picture in the following chapter (Prince et al., 2015).

The neuropathology of AD is characterized by progressive tissue atrophy, especially in the hippocampus, entorhinal cortex, and medial temporal cortex (Chetelat & Baron, 2003). As the disease progresses, the association cortex is also affected (Braak & Braak, 1991; Braak et al., 1998). Histologically, extracellular β-amyloid plaques (Glenner & Wong, 1984), also called senile

plaques (SP), as well as intracellular neurofibrils of hyper-phosphorylated tau-protein are characteristic (Golde et al., 2000; Mattson, 2004). Protein deposits can evoke the loss of synapses and subsequent cell death of neurons, although the exact mechanism is still unclear (Scheff & Price, 1993). Modifications of membrane receptors or mitochondria are discussed (Lin & Beal, 2006; G. M. Shankar et al., 2007). Both β-amyloid and tau-proteins occur physiologically in neurons. The main forms of β-amyloids are the two isoforms Aβ-40, consisting of 40 amino acids and Aβ-42, composed of 42 amino acids (Haass & Selkoe, 1993). The pathomechanism of plaque formation describes a splitting of the amyloid precursor protein (APP) into β-amyloid by the β-secretase. Subsequently, the poorly soluble β-amyloids accumulate into plaques (Cameron & Landreth, 2010). In the following course of the disease, a mismatch between the overproduction of β-amyloids and cellular regulatory mechanisms such as autophagocytosis by lysosomes increases (Bendiske & Bahr, 2003). The causes have not yet been conclusively clarified. A gene mutation of the risk allele for apolipoprotein E4 (apoE4), which inhibits the degradation of β-amyloid, seems to be associated with AD progress (Kim et al., 2009), (Dermaut et al., 2001).

The usual clinical diagnosis includes a comprehensive psychiatric personal anamnesis and an extern anamnesis of a person close to the patient. Moreover, a neuropsychological examination, cognitive testing, neuroimaging procedures (computer tomography (CT), magnetic resonance imaging (MRI)), and a laboratory diagnosis, supplemented with cerebrospinal fluid analysis for the determination of β-amyloid and total tau (Deutsche Gesellschaft für Neurologie, 2016) are investigated. In the clinical diagnosis of Alzheimer's dementia, the following symptoms are evaluated according to ICD-10 (2019): it is a chronic progressive disease of the brain with disorders of cortical functions such as abstract thinking, memory, ability to learn, ability to judge, information processing, language, changes in social behavior (emotional instability, apathy, anxiety, aggressiveness) without clouding the consciousness (World Health Organization, 1992). The symptoms have to persist over at least six months, and daily abilities have to be disturbed.

The therapeutic possibilities of AD are currently still limited. Complete healing cannot be achieved, which is why the treatments are limited to a reduction of disease progression. For drug treatment, acetylcholinesterase inhibitors (compensate for the acetylcholine deficiency) and the NMDA (N-Methyl-D-Aspartate) receptor antagonist Memantine (inhibits the overactivation of the NMDA receptor and thus the pathological effect of excess glutamate) are used, which help to delay the course of the disease by one to two years (Gutzmann & Mahlberg, 2009; Scarpini et al., 2003), particularly the cognitive symptoms. Neuroleptics are used in the later stages of the disease for the treatment of psychotic symptoms or agitation and aggression. Besides, vigilance-increasing nootropics are used alternatively (Rommelspacher, 2002) without scientific proof. Summing up, the main risk factor for AD is age (Hebert et al., 1995). In addition, the genotype apoE4 is associated with an increase in risk (Bertram & Tanzi, 2008). Further suspected risk factors are systemic pre-existing conditions such as hypertension, type 2 diabetes, depression, strokes, craniocerebral trauma, hearing loss, sleep disturbance, increased cholesterol levels but also an unhealthy lifestyle with a lack of physical exercise and social interaction, increased alcohol consumption, a high-fat diet, and a low level of education (de Oliveira et al., 2014; Edwards Iii et al., 2019; X. Li et al., 2015; Middleton & Yaffe, 2009; Roh et al., 2012; Thomson et al., 2017; Verghese et al., 2003; Vijayan & Reddy, 2016).

1.2.3. Prodromal markers of neurodegeneration

It is well known that neurodegenerative processes begin more than a decade before the onset of the first clinical symptoms of neurodegenerative diseases such as Alzheimer's (AD) or Parkinson's Disease (PD) (Postuma et al., 2010; Price & Morris, 1999). Many supposedly unspecific symptoms can therefore act as early warning markers (Berg, 2008). Popular examples are REM Sleep Behavior Disorder (RBD), depression, and idiopathic hyposmia. With regard to PD, irregularities in neuroimaging (positron emission tomography (PET)), which indicate a change in the substantia nigra, and slight motor abnormalities also

provide first indications (Berg, 2008). The course of AD is characterized by a preclinical phase lasting up to several years, followed by a transition phase, mild cognitive impairment (MCI), to a clinical diagnosable picture (Petersen, 2016). MCI is characterized by slight cognitive impairments, which, however, do not yet affect an independent life. Patients with MCI tend to develop AD at a rate of 8-15% per year (Grundman et al., 2004; Petersen, 2016). In the preclinical phase of AD some prodromal markers are already detectable. These are biomarkers that can be detected as changes in cerebrospinal fluid (CSF), blood serum or olfactory perception (Berg, 2008; Forlenza et al., 2010; Hawkes, 2006). In the disease progression of AD, CSF reveals a decrease of 42 amino acid isoform of β-amyloid-protein (Aβ-42) and an increase of hyper-phosphorylated tau (p-tau181) and the total amount of tau (T-tau) (Mouton-Liger et al., 2014; Rosén et al., 2013). Since biomarkers in blood serum are easier to examine, this method is preferred to the determination of CSF. However, the concentration of brain-derived proteins in the blood is significantly lower than in CSF due to the blood-brain barrier. In addition, a strong dilution of the proteins in the plasma volume can blur the results. Further, the proportion of endogenous antibodies in the blood is much higher than in cerebrospinal fluid. Some antibodies can react erroneously with the immunological test substance for measuring the biomarker and thus falsify the results. Nevertheless, as the methods for analyzing the most promising blood markers (the Aβ-42/Aβ-40 ratio, tau, and plasma neurofilament light (NfL)) become more sensitive, the procedure might soon establish in primary diagnostic screening to identify early neurodegeneration (Khalil et al., 2018; Weston et al., 2017; Zetterberg & Burnham, 2019).

Further prodromal markers can be determined using neuroimaging methods, but the exact differentiation of physiological and pathological remodeling processes poses a challenge. In addition to the general atrophy of the brain volume, volume decreases in the medial temporal lobe, entorhinal cortex, and hippocampus allow predictions to be made about the severity of the disease (Korf et al., 2004). Using functional imaging (fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET)), a reduction of glucose metabolism in the frontal, parietotemporal, and posterior cingulate cortices was detected as a sign of early AD (Mosconi,

2005). Also a reduction of cerebral blood flow, determined by arterial spin labeling magnetic resonance imaging (ASL-MRI), has established itself as a sensitive prodromal marker for neurodegeneration (Hays et al., 2016). Newer methods of in vivo AD diagnostics are amyloid-PET and tau-PET. For amyloid-PET radioactive fluorine isotopes ^{18}F as Florbetapir, Florbetaben, and Flutemetamol are available as tracers for Aβ (Anand & Sabbagh, 2017; Daerr et al., 2017; Suppiah et al., 2019). In addition, PET imaging also includes several ^{18}F tracers that detect tau-proteins (e.g. [^{18}F]MK-6240) (Leuzy et al., 2019). Both methods became established in clinical diagnostics only a few years ago. They provide important insights into the as yet unanswered question of whether tau and amyloid independently or synergistically cause cognitive decline and about their connection to other pathologies.

1.2.4. Ageing and sex differences

The current state of research assumes a gender-dependent brain development in old age. Genetics and the endocrine system are regarded as the main influencing factors. The sex hormones, in particular the androgen testosterone, are attributed a central role. According to current knowledge, it is assumed that testosterone influences structural changes of the hypothalamus, the hippocampus, and the amygdala (Filova et al., 2013). The testosterone metabolism enables action at androgen receptors via conversion by 5-alpha-reductase to dihydrotestosterone (DHT) and interaction at estrogen receptors via conversion to estradiol (E) via aromatase. Testosterone unfolds its effect in both sexes but is present in men in significantly higher concentrations. In a transgender hormone therapy study, it could be shown that the substitution of estrogens and anti-androgens causes a reduction of the male brain towards a female brain size, while the intake of androgens led to an increase in brain volume (Pol et al., 2006). A possible correlation between brain volume and testosterone levels is also indicated by the observation that older men show a stronger reduction in brain volume than women (Armstrong et al., 2019; Curiati et al., 2009; Xu et al., 2000), which is accompanied by an increased reduction in testosterone

levels. This effect was mainly detected in the dorsolateral prefrontal cortex (dlPFC) (Raz et al., 1993). The age-dependent increase of the peripheral cerebrospinal fluid volume (CSF-volume), as a sign of cortical atrophy, is also stronger in men than in women (Coffey et al., 1998).

Various associations between sex hormones, behavioral data, cognition and changes in the area of the prefrontal cortex were found (Keenan et al., 2001; Schoning et al., 2007). An fNIRS study by Li et al. (2010) confirmed higher effectiveness of women in a visual working memory task compared to the male participants in the comparison of behavioral data and cortical activation in the PFC. Although both sexes performed the same, the women showed a significantly lower hemodynamic response (T. Li et al., 2010). Morley et al. (1997) analyzed testosterone levels and cognitive performance in a sample of 56 men aged from 21 to 84 years and found that age-related decreases in hormone levels predicted a decrease in visual and verbal memory. The differences between male and female age development intensify after the onset of female menopause due to the extensive hormonal changes in women (Kantarci et al., 2018).

In addition to hormonal differences, external gender-specific factors influencing lifestyle are also discussed. An important factor is alcohol consumption. Numerous studies on consumer behavior and cultural influences prove an increased lifelong alcohol consumption among men compared to women (Almeida-Filho et al., 2004; Parry et al., 2005; Wilsnack et al., 2000). A study by Pfefferbaum et al. (2001) found age-dependent differences in morphology between alcoholic brains. The brains of male alcohol consumers showed age effects in the prefrontal grey matter and the sulcus volume, whereas in women the lateral ventricles were more affected (Pfefferbaum et al., 2001).

1.3. Diagnosis options for cognitive diseases

1.3.1. Trail Making Test (TMT)

The Trail Making Test (TMT) is an internationally recognized and widespread neuropsychological screening method for recording brain function performance (Reitan, 1992). TMT examines various parameters such as working memory,

mental flexibility, visuomotor processing speed, and executive functions (Arbuthnott & Frank, 2000). Meanwhile, there are voice-controlled, computer-controlled or written versions of the TMT. In the present study, the written TMT was applied according to the standards of the neuropsychological test battery Consortium to Establish a Registry for Alzheimer's Disease (CERAD) -Plus (Morris et al., 1988). The German-language version of the CERAD test was created at the University Hospital in Basel and extended to the CERAD-Plus version by the addition of the Trail Making Test and the phonematic fluid test (Schmid et al., 2014; Thalmann & Monsch, 1997).

The "paper-pencil"-TMT is characterized by its simple handling and can be easily and reliably integrated into the clinical routine. A decisive advantage over other test procedures is that no movement artifacts are provoked by the activation of the mimic musculature when speaking, as this is a purely written test.

The classic version of TMT consists of two sub-tests, TMT-A and TMT-B. In TMT-A, the participants are instructed to combine numbers distributed randomly on a sheet in ascending order as quickly as possible. In the TMT-B, numbers and letters must be linked alternately according to the ascending number series and the alphabet. TMT induces cortical activation in the cognitive control network (CCN). In the sub-test TMT-B, the executive frontal cortices such as the inferior and middle frontal cortex, dorsolateral prefrontal cortex (dlPFC) and cingulate gyrus are activated (Hagen et al., 2014; Jacobson et al., 2011). In addition to visuomotor processing speed and working memory, the TMT-B also requires mental flexibility and task-switching skills (Sanchez-Cubillo et al., 2009). Due to the divided attention, TMT-B is considered to be the more challenging task and therefore increased blood oxygenation can be expected during TMT-B execution. Originally, the time to completion of the respective TMT sub-test was determined, but meanwhile, there are also test designs with time-limited measurements, where the number of processed items reflects inter-individual performance differences (Hagen et al., 2014).

In addition to neuropsychological symptoms, influencing factors such as age, education, and occupation can also distort the results of TMT (Amodio et al., 2002).

1.3.2. Functional Near-infrared Spectroscopy (fNIRS)

Functional near-infrared spectroscopy (fNIRS) is a non-invasive monitoring method first developed by Jobsis for the medical field in 1977 (Jobsis, 1977). Since then, the technology based on the measurement of cerebral oxygen saturation has been gaining ground in various medical fields. In neuroscience, fNIRS has proven to be a reliable alternative neuroimaging method to functional magnetic resonance imaging (fMRI), as the determination of concentration changes of oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin allows conclusions to be drawn about cortical activation (Colier et al., 1995). The measurement method is based on the principle of neurovascular coupling. This means that the increased energy requirement resulting from cerebral activity is covered by a local increase in blood flow with an increased concentration of oxygenated hemoglobin (O_2Hb) and a reduced concentration of deoxygenated hemoglobin (HHb). Accordingly, an indirect measurement of neuronal activity is performed via increased metabolism and oxygen consumption. The ratio changes between O_2Hb and HHb as well as the changes in absolute blood flow can be measured as an indirect correlate of neuronal activity (Villringer et al., 1993).

Light from the near-infrared spectrum (650 to 1000 nm wavelength) penetrates biological tissue well and is absorbed to varying degrees by the individual tissue structures (bone, fat, collagen, cerebrospinal fluid, hemoglobin) (Delpy & Cope, 1997). This wavelength range is therefore called the "optical window". The brain tissue has relatively constant scattering and absorption properties, which is why changes in hemoglobin concentration over time can be easily detected. The dominant factor in tissue penetration is scattering, which is 100 times more likely than absorption (Delpy & Cope, 1997). The near-infrared light is mainly attenuated by the chromophores O_2Hb and HHb. The absorption spectrum of hemoglobin depends on the degree of oxygenation. Therefore, the two chromophores O_2Hb and HHb can be differentiated according to their absorption spectra (Haeussinger et al., 2011; Obrig & Villringer, 2003).

The technical principle is based on the emission of near-infrared light (photons) under the skullcap by emitters. After tissue-dependent scattering and absorption,

the reflected part is captured by detectors arranged at a distance of up to three centimeters around the emitter (Ferrari & Quaresima, 2012). According to the modified Lambert-Beer law, it is possible to calculate the concentration changes of O_2Hb and HHb from the ratio of emitted and detected light. Together emitter and detector form an "optode" and are combined as an fNIRS channel. Due to the scattering, no absolute values of the hemoglobin concentration can be determined. Conclusions on brain activation can therefore only be drawn from relative concentration changes between different measurement conditions (rest vs. task). Since the maximum penetration depth of near-infrared light is about two to three centimeters (Haeussinger et al., 2011), fNIRS can only detect hemodynamic changes within the cerebral cortex.

Compared to other neuroimaging methods (functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), scintigraphy, positron emission tomography (PET)), fNIRS offers some decisive advantages in neuropsychology: similar to fMRI, superficial cortical activations and the duration, intensity, and spatial extent of neuronal processes are recorded. In contrast to fMRI, which is performed lying in a narrow and noisy scanner, fNIRS allows measurements under ecologically valid and stress-free conditions, in an upright sitting position and during social interaction. The clinically simple handling is characterized by the high mobility of the fNIRS device and its cost-effective operation (Ferrari & Quaresima, 2012). Thus, measurements are also possible with physically and mentally restricted persons, claustrophobics, and children. Another advantage is that fNIRS is relatively insensitive to motion artifacts, and can therefore also be used to perform cognitive tasks (Ehlis et al., 2014; Fallgatter et al., 2004; Lloyd-Fox et al., 2010). Finally, the technology ensures a high temporal resolution, which enables the direct assignment of a hemodynamic response to neuronal activity (Ehlis et al., 2014). Over the past few decades, fNIRS has also established itself in intensive care medicine and anesthesia for monitoring cerebral oxygen saturation (Kirkpatrick, 1997).

Besides the significant advantages, fNIRS also has some negative aspects. Disadvantages are the limited spatial resolution, the reduced penetration depth of only two to three centimeters (Haeussinger et al., 2011), and possible

distortions due to anatomical conditions (Irani et al., 2007). Another point of criticism is the more difficult distinction between cerebral and extracerebral changes in blood flow. Extra cerebral fluctuations in the blood circulation of the skin through temperature changes, pain, stress or in the blood circulation of the skeletal muscles due to activation of the mimic or chewing muscles can erroneously be interpreted as cerebral signals. A particularly strong influence is attributed to the mimic muscles M. corrugator supercilii and M. frontalis as well as the masticatory muscle M. temporalis (Boas et al., 2004; Haeussinger et al., 2011; Schecklmann et al., 2010).

1.3.3. Functional Connectivity

As stated in the introduction, changes in functional brain organization are considered to be particularly sensitive markers of aging processes. It is even hypothesized that functional changes precede structural changes (Jack et al., 2010). In this dissertation, the aspect of functional connectivity (FC) was examined regarding its significance for age-related changes. The following chapter explains the concept of FC.

First described by Friston et al. (1993), the term functional connectivity (FC) is defined as the temporal relationship between spatially separated neurophysiological processes. In this way, different networks of the brain are created which are functionally connected during certain processes. It is assumed that these functional connections between different brain areas exist both at rest and during task accomplishment. From the research results to date, functional networks have been defined such as the default mode network (DMN), cognitive control network (CCN), dorsal attention network (DAN), and salience network (SN). There are often similarities between FC and the structural anatomical connections via nerve fibers.

The simplest method of determining FC is to calculate temporal correlations between two different brain regions. Mathematically, FC is equal to the Pearson correlation coefficient of two time series (xt and yt) of two different brain regions:

$$corr\ (x, y) = \frac{\sum_{i=1}^{n}(xi - \overline{x}) * (yi - \overline{y})}{\sqrt{\sum_{i=1}^{n}(xi - \overline{x})^2 * \sum_{i=1}^{n}(yi - \overline{y})^2}}$$

Formula 1: Pearson-correlation

The correlation is undirected, therefore no causal relationships (e.g., does region A influence region B or does region B influence region A?) are represented. The type of causal relationship is described by means of effective connectivity, which allows conclusions to be drawn about the causality of the connections (Eickhoff & Grefkes, 2011).

Meanwhile, several methods for the analysis of FC have been established. The seed-based method uses a seed voxel as a starting point from which the time series of all other brain voxels are correlated after extraction of the resting-state reference time series (Andrews-Hanna et al., 2007; B. Biswal et al., 1995). Thus, a FC-map is generated which reflects the functional connections of an a priori defined brain region (seed) (B. B. Biswal et al., 1997). The disadvantage is that only one pre-defined seed region and its functional connections are recorded, and a global analysis of the entire brain organization is not possible. In order to investigate the connectivity of the entire brain without a pre-defined seed region, model-independent methods such as clustering (Cordes et al., 2002), principal component analysis (PCA) (Friston et al., 1993), and independent component analysis (ICA) (Beckmann et al., 2005) have proved their worth. In particular, the latter is frequently used and is based on the principle that a large number of underlying spatial sources of resting-state signals are searched for that are maximally independent of each other (van den Heuvel & Hulshoff Pol, 2010).

Effective connectivity, on the other hand, which describes causal relationships between the brain regions, can be determined using regression models (psychophysiological interaction analysis (PPI)) (Friston et al., 1997), dynamic causal modeling (DCM) (Friston et al., 2003) or time series models (e.g. Granger causality) (L. Harrison et al., 2003).

In the meantime, FC in the resting-state (rsFC) has proven to be an indicator of structural connectivity patterns in addition to the analysis of functional brain organization. Biswal and his colleagues were the first to demonstrate that at rest the fMRI Blood-Oxygenation-Level Dependent (BOLD) time series of the right and left hemisphere of the primary motor network are highly correlated (B. Biswal et al., 1995). From this, it was deduced that even at rest, brain areas functionally interact via correlating spontaneous low-frequency fluctuations in the BOLD signal (<0.1 Hz) (Greicius et al., 2009). In the following years, in addition to resting state networks, also functional networks could be identified which were related to the execution of specific tasks. Among the most important are the default mode network (DMN), the cognitive control network (CCN), the salience network, the affective fronto-limbic network, and the dorsal attention network (DAN). The DMN is most active at rest and is associated with the process of self-reflection. The CCN, on the other hand, is active in processes of increased attention such as decision making, planning, and memory tasks. CCN consists of the areas of the dorsolateral prefrontal cortex (dlPFC), posterior parietal cortex (PPC), dorsal anterior cingulate cortex (ACC), anterior insula cortex (AIC), and inferior frontal junction (IFJ) (Cole & Schneider, 2007).

1.4. Intermediate Summary

In the previous sections, I provided an overview of cerebral physiological and pathological aging processes and presented diagnostic options. The cognitive test used in this work, the Trail Making Test, and the applied neuroimaging method of functional near-infrared spectroscopy, were examined more closely. Finally, the principle of functional connectivity for the detection of brain organization and the relevant networks were also investigated.

As explained above, different functional and structural changes of the brain take place in old age. The extent to which these evoke cognitive decline, and whether the body's own compensation mechanisms counteract the reduction in performance, was the subject of various research groups. Research on the neuronal basis of ageing processes in brain activation and coupling may have

the potential to provide new insights in this field in order to detect neurodegeneration already in the preclinical stage. In the following chapters, the current neurophysiological state of scientific knowledge will be presented.

1.5. Background for the present projects

1.5.1. Relations of ageing, performance loss, and compensatory effects

The clinical picture of ageing processes is subject to great variability. A fundamental question of cognitive ageing research is still why some individuals lose their cognitive abilities with advancing age, while others can maintain sufficient cognitive capacities for an independent life. Today's generally accepted view is based on a biological focus. Social and ecological factors affect cognition by influencing biological processes.

At the cognitive level, ageing is associated with a decrease in executive functions, working memory, attention, mental flexibility, working speed, and long-term memory (Crawford et al., 2000; Hedden & Yoon, 2006; T. A. Salthouse, 1996). In addition, cognitive processes are slower in general (T. A. Salthouse, 1996). These observations justify the research focus on the identification of correlations between neuronal and cognitive changes. The relationship between brain and cognition is dynamic and subject to lifelong change, which makes mutual weighting difficult. However, the relationship is undisputed, and the investigation of the underlying mechanisms is essential for a better understanding.

The impairment of basic executive functions leads to a loss of performance, especially in the performance of tasks. However, there are considerable differences in performance between individuals (Sliwinski & Buschke, 1999) and also within the individuals, the limitations can vary greatly and occur very locally only in special cognitive domains. For example, only episodic and not executive memory can be affected and vice versa (Glisky et al., 1995). This is interesting because certain independence becomes apparent despite strong interactions of the different cognitive areas. In the Berlin Age Study (BASE) the performance

variability of 516 older participants was recorded in a longitudinal comparison over 7 years (Mayer & Baltes, 1999). Among other things, memory performance, speed of perception, thinking ability, and word fluency were tested, whereby a negative correlation between age and performance became clear. Yet, a pronounced inter-individual variability was observed (Mayer & Baltes, 1999).

Various theories offer explanatory approaches for cognitive development in old age. One characteristic of cognitive ageing is slower information processing (T. A. Salthouse, 1996). Salthouse (1996) hypothesized that the processing speed and cognition are causally related, since a reduced processing speed makes the simultaneous execution of cognitive processes more difficult and, due to the time delay, results from previous processes can no longer be linked to current influences. Another theory assumes a limited capacity of the working and sensory memory, which decreases further with age and may even reach its limit (Neath, 1998). The so-called inhibition theory of Hasher and Zacks (1988) describes an inefficiency of the brain's inhibition processes with a decrease in the ability to suppress irrelevant information and an associated increase in the susceptibility of memory processes to disruption (Hasher & Zacks, 1988; Hasher et al., 1999). The common cause hypothesis attributes the age-related deficits of cognitive, sensory, and sensorimotor resources to a common neurobiological mechanism (Baltes & Lindenberger, 1997). It predicts an increasing interaction between cognition and sensorics with increasing age and simultaneously decreasing cognitive competence. The neurophysiological correlates of these competencies are structural and functional changes of brain organization, which are described in more detail in chapters 1.2.1 and 1.2.2.

The affected cognitive functions will be discussed in the following section. The most strongly affected by age effects is the working memory (Babcock & Salthouse, 1990; Hasher & Zacks, 1988). A popular hypothesis is that working memory impairment is the source of several other cognitive deficits in the field of decision making, problem solving, language, and long-term memory (D. C. Park et al., 2002; Timothy A. Salthouse, 1991). Information processing in the working memory is mainly controlled by sections of the prefrontal cortex (PFC), whereby

the left hemisphere is assumed to be more important in verbal tasks and the right hemisphere in visuospatial tasks (Wager et al., 2003).

Cerebral aging processes also affect attention. Attention is an indispensable essence of any everyday task unless it is an automated and familiar process (McDowd & Shaw, 2000). The concept of attention has been further subdivided in many ways according to theory, whereby selective, sustained, and divided attention have established themselves as important definitions. Selective attention is characterized by the ability to respond to relevant stimuli while ignoring surrounding disruptive influences. An experimental psychological test example is the Stroop task (Stroop, 1935). For this test, the participants are instructed to name color words that do not correspond to their color representation (e.g., the word blue is printed in red). Although the older participants showed a reduction in processing speed, they were not affected by increased distraction, which is why no general conclusions can be drawn about disturbed selective attention (McDowd & Shaw, 2000). However, direct age influences seem to exist in the area of divided attention. Divided attention is always relevant when two or more stimuli have to be perceived contemporaneously, and different information sources have to be processed simultaneously (Moriarty, 2015). Divided attention can be investigated using dual-task and task-switching tasks such as the Trail Making Test B (TMT-B). TMT-B execution requires attention to be switched between different subtasks. The results of different dual-task test series agree on the assumption that older individuals have greater difficulties in solving tasks that require divided attention than younger individuals (Tsang & Shaner, 1998). It is unclear whether this phenomenon can be explained by the age-related decline in cognitive resources, which becomes particularly apparent when solving complex tasks since the already limited attention has to be distributed over several stimuli. Sustained attention requires, as the name suggests, maintaining concentration on a task over a longer period, as measured by so-called vigilance tasks. Interestingly, older people usually do not show any deficits with regard to these requirements.

There is evidence that perception, a complex process of stimulus absorption and information processing, is also reduced in older adults (Schneider & Pichora-

Fuller, 2000). However, such results should be viewed with caution, as the mutual influence of sensory perception and cognition has not been conclusively clarified. Often, supposed cognitive differences in age studies are only due to peripheral impairments such as hearing and vision loss (Baltes & Lindenberger, 1997).

Age research also focuses on long-term memory since the effects of limitations become apparent early in everyday life. Long-term memory is the memory that is used to retrieve information and events from a long time ago. Its central components are episodic memory, semantic memory, and procedural memory. The strongest age influence is assumed to be in episodic memory, the memory for autobiographical events (Nyberg et al., 2012; Shing et al., 2010). This can be due to poor coding, difficulties in consolidating or retrieving events. The coding describes the storage of new information and is significantly associated with the prefrontal cortex. If deficits occur there, new information is stored more poorly, which in turn causes difficulties in retrieving the information (Craik et al., 1983). The consolidation phase is the time interval that should lead to permanent storage of information, but at the same time is still susceptible to memory loss (Jennings & Jacoby, 1997). Changes in the hippocampus of older adults have been identified as a neurophysiological correlate for deviant memory consolidation (Harand et al., 2012). Parkin and Walter (1992) associated retrieval difficulties in older adults with frontal dysfunction. A second component of long-term memory is semantic memory, which in contrast to episodic memory is less associated with age restrictions (Nyberg et al., 1996). Semantic memory is basic knowledge from general education and the worldview. Furthermore, procedural memory, which stores automated abilities such as walking and cycling, seems to be less affected by ageing processes as well (Churchill et al., 2003).

The considerable inter-individual heterogeneity of ageing processes was tried to explain by lifestyle differences on the one hand and individual neurophysiological compensation mechanisms on the other. There is evidence of a stronger bilateral prefrontal activation in old age, which accumulates in the so-called Hemispheric asymmetry reduction in old adults-model (Harold-model) (Cabeza, 2002). The age-related reduction of asymmetry can be interpreted as a compensation

mechanism by reorganization as well a dedifferentiation and sign of inefficiency (Cabeza, 2002; Logan et al., 2002).

At the structural level, a proliferation of dendritic extent has been reported as a compensatory response to neuronal loss (Coleman & Flood, 1986). Furthermore, there is evidence of age-related changes in the brain associated with the reinforcement of existing structures or the creation of an additional scaffolding: the so-called scaffolding theory of aging and cognition (STAC) (D. C. Park & Reuter-Lorenz, 2009). STAC argues that increased frontal activation in old age is an indication of an adaptive brain that develops compensatory scaffolds to counteract the decrease in neuronal numbers (D. C. Park & Reuter-Lorenz, 2009). Finally, the "reserve capacity" of the brain is often equated with a compensatory influence. However, the term reserve capacity describes the premorbid state of the brain, which can be modeled, for example, by an increased synaptic density or redundancy through an increased educational level, whereas compensation can only occur after the occurrence of cognitive deficits (Montine et al., 2019).

1.5.2. Studies showing altered activity in elderly

The reorganization of the aging brain becomes apparent in an altered cortical activity. According to the Hemispheric asymmetry reduction in old adults model (HAROLD model) (Cabeza, 2002), prefrontal activation in cognitive tasks appears less lateralized in older compared to younger participants. Cabeza et al. (2002) hypothesized their discovery as compensation since increased bilateral activation was seen in older high-performing participants, whereas low-performers showed a unilateral activation pattern. Cabeza's findings of strong bilateral activation in old age were also confirmed by other research groups (Muller et al., 2014; P. A. Reuter-Lorenz et al., 2000; Rossi et al., 2004). An fNIRS study by L. D. Muller et al. (2014) found a stronger bilateral activation in the dorsolateral prefrontal cortex (dlPFC) in older adults compared to younger adults. However, the compensation hypothesis was also critically questioned, since bilateral activation in older subjects does not necessarily have to reflect age

compensation, instead, it can also be interpreted as a sign of increased stress in challenging circumstances caused by the cognitive task with additional recruitment from the contralateral hemisphere (Höller-Wallscheid et al., 2017; Ivarez Merino et al., 2018).

In addition to asymmetry reduction, an association between overactive brain regions and performance success in older people was also found (Rossi et al., 2004). In an fMRI study by Methqal et al. (2017), a stronger involvement of the frontal regions with increasing demands in the execution of a word-semantic-rule-based task from the Wisconsin Sorting Card Test in older volunteers became clear. After reaching a certain requirement level, however, a declining effect with decreasing activation was observed (Methqal et al., 2017).

An fMRI study by Grady et al. (2006), which used encoding and recognition tasks to compare participants from three different age groups, also provided exciting insights into the brain's reorganization. Surprisingly, they found a linear increase in activation with age in regions of the default mode network that are usually reduced during task execution. In contrast, the area of dlPFC, which is typically associated with task accomplishment, showed a decrease in activation with increasing age (C. L. Grady et al., 2006). The balance shift can be interpreted as the inability of older participants to access the same cognitive resources as younger participants, and as increased distractibility.

In addition to the recording of cortical blood oxygenation, an fNIRS study by Zeller et al. (2019) also showed spontaneous low-frequency oscillations (LFO) as an indicator of cognitive changes. Both, persons with mild cognitive impairment and older persons showed fewer LFOs in the frontal cortex than younger, healthy participants. Interestingly, their results revealed a negative correlation of worse performance, which was shown in longer response times to the attentional performance test (TAP) and increased LFO in parietal regions.

1.5.3. Studies showing altered FC in elderly

The investigations of cortical activation and functional connectivity (FC) should be complementary, as they provide different information about the underlying

cerebral processes. While activation acts as a direct correlate of neuronal metabolism, FC shows functional correlations in the activation and deactivation of different brain regions. The following section is dedicated to the presentation of the current state of studies regarding the effects of ageing on functional connectivity. As shown above, intra- and internetwork (within and between) connections are considered.

The majority of the research results confirm indications of an age-related change in resting state FC (rsFC). The most reliable consistencies showed a decrease in functional connectivity within resting state networks such as the default mode network (DMN) and the salience network (SN) (Chan et al., 2014; Ferreira et al., 2016; Geerligs et al., 2015; C. Grady et al., 2016). On the contrary, FC increases were reported for between network FC (Chan et al., 2014; Ferreira & Busatto, 2013; Ng et al., 2016). Other studies revealed increased FC in the frontal cortex with advancing age in conjunction with maintenance of performance (Davis et al., 2012; Yang et al., 2009). According to the contents presented in chapter 1.3.3, there is a multitude of FC measurement methods, which makes direct comparability of the results difficult. A recently published study by Varangis et al. (2019) combined various resting state FC analysis methods to provide a comprehensive overview of functional brain organization in old age. Their central results showed an age-related global reduction of intranetwork connectivity and lower system segregation (Varangis et al., 2019). The age effects concerning an increase of between network connections mentioned in earlier studies (Betzel et al., 2014; Chan et al., 2014) could not be reproduced by Varangis et al. (2019).

In addition to the division into intra- and internetwork FC, a more exact localization of the rsFC changed with age seems indispensable. In the past, studies specified a reduced FC in old age within the DMN (Andrews-Hanna et al., 2007; Chan et al., 2014; Ferreira et al., 2016; Geerligs et al., 2015; C. Grady et al., 2016), frontoparietal (Betzel et al., 2014; Geerligs et al., 2015), dorsal attention (Tomasi & Volkow, 2012), and cingulo-opercular cortex (Geerligs et al., 2015). An age-related increase in FC was found in the somatomotor cortex (Song et al., 2014; Tomasi & Volkow, 2012). Moreover, a resting state FC measurement in a group of healthy middle-aged adults revealed a negative correlation of FC between

amygdala and dorsal medial prefrontal cortex (dmPFC), and between amygdala and somatomotor cortex with age (Xiao et al., 2018).
In the past, FC has also proven to be a predictor of neurodegenerative changes. It has been confirmed several times that FC in patients with mild cognitive impairment (MCI) or Alzheimer's disease (AD) differs from FC in healthy subjects (Binnewijzend et al., 2012; Dai et al., 2014; Sorg et al., 2007; Wang et al., 2007). Yang et al. (2009), for example, exposed in a fMRI study that FC between bilateral dlPFCs was elevated in patients with MCI compared to older, healthy controls. Interestingly, the two groups did not differ in cortical activation and behavioral performance data (Yang et al., 2009).

1.6. Aims and questions of the studies

This dissertation is intended to make a supplementary contribution to a better understanding of the research findings, presented in the previous sections regarding age-related cortical restructuring. A total of three studies were conducted to assess age-related changes in cognitive performance, cortical activation, and functional connectivity.
For the reasons presented in chapter 1.3.2, fNIRS was used to measure cortical blood oxygenation. Age differences in cognitive and executive functions were investigated using TMT.
Based on the current state of research, the following questions arose:

Study 1*: Comparison of speed versus complexity effects on the hemodynamic response of the Trail Making Test in block designs*
Study one was devoted to the investigation of the influences of working speed and task complexity on blood oxygenation, to fundamentally verify the validity of the use of study designs that integrate TMT in block designs.

Research question 1: Are there differences between the influence of working speed (slow-medium-fast) and task complexity (TMT-A vs. TMT-B) on the hemodynamic response?

Research question 2: Does the use of a time-limited block design level performance differences in TMT?

Study 2: *Age-related deterioration of performance and increase of cortex activity comparing time- versus item-controlled fNIRS measurement*

In the second study, we focused on the effects of age differences on cortical activation in the area of CCN during the execution of the cognitively demanding TMT. From the findings of study one that both working speed and task complexity influence cortical oxygen saturation, we developed an additional variable, the blood oxygenation per solved item, as we considered it particularly sensitive to age-related performance reductions.

Research question 3: Are there age differences in TMT performance (number of processed items)?

Research question 4: How do age groups differ concerning the cortical blood oxygenation during task performance (time-controlled)?

Research question 5: How do the age groups differ with regard to cortical blood oxygenation per solved item (item-controlled)?

Research question 6: Can age-related compensation mechanisms be assumed?

Study 3: *Effects of ageing on functional connectivity. A comparison of resting state versus task measurement using fNIRS*

The third study is based on the assumption that changes in functional brain organization are particularly sensitive to early age effects. We particularly weighted the differences in functional connectivity of older adults at rest and during TMT completion. Intra- and internetwork connections within the CCN and DAN were recorded.

Research question 7: Does functional connectivity (FC) differ from older and younger participants during the resting state measurement and task performance? If so, what region of interest (ROI) would be affected?

Research question 8: Are age effects more visible during task performance than during resting state measurements?

1.7. Hypotheses

- Both working speed and task complexity have an influence on blood oxygenation. For this reason, the use of a block design in the TMT can be problematic if the number of items solved will not be integrated.

- With older age, cognitive decline leads to increased processing times and compensatory heightened cortical blood oxygenation.

- TMT-A and TMT-B require a higher cerebral activity than the motor control task TMT-C.

- TMT-B requires an increased cortical activity compared to TMT-A due to the necessity of task-switching.

- TMT-B is considered to be the more difficult task, therefore fewer solved items are to be expected for TMT-B.

- Older people could compensate for performance deficits by slowing down their working speed. The integration of an additional variable into the analysis, the cortical blood oxygenation per solved item, is a valid approach to consider this component.

- Functional connectivity is a particularly sensitive marker for early age-related brain reorganizations. Due to the increased performance

requirement, age effects are more pronounced in the FC in task accomplishment than in the resting state FC.

2. Results

2.1. Study 1 – Comparison of speed versus complexity effects on the hemodynamic response of the Trail Making Test in block designs

The contents of this chapter are published:

David Rosenbaum[1], Leonore Blum[1], Paul Schweizer[1], Andreas J. Fallgatter[1,2,3], Martin J. Herrmann[4], Ann-Christine Ehlis[1,3], Florian G. Metzger[1,5], “ Comparison of speed versus complexity effects on the hemodynamic response of the trail making test in block designs,” *Neurophoton*. 5 (4), 045007 (2018), doi: 10.1117/1.NPh.5.4.045007.

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Keywords: functional near-infrared spectroscopy; trail making test; processing speed; task complexity.

Abstract

The use of functional near-infrared spectroscopy (fNIRS) in block designs provides measures of cortical activity in ecologically valid environments. However, in some cases, the use of block designs may be problematic when data are not corrected for performance in a time-restricted block. We sought to investigate the effects of task complexity and processing speed on hemodynamic responses in an fNIRS block design. To differentiate the effects of task complexity and processing speed, 20 subjects completed the trail making test (TMT) in two versions (TMT-A versus TMT-B) and three different speed levels (slow versus moderate versus fast). During TMT-A, subjects are asked to connect encircled numbers in numerically ascending order (1-2-3…). In the more complex TMT-B, subjects are instructed to connect encircled numbers and letters in alternating ascending order (1-A-2-B…). To illustrate the obscuring effects of processing speed on task complexity, we perform two different analyses. First, we analyze the classical measures of oxygenated blood, and second, we analyze the measures corrected for the number of processed items. Our results show large effects for processing speed within the bilateral inferior frontal gyrus, left dorsolateral prefrontal cortex, and superior parietal lobule (SPL). The TMT contrast did not show significant effects with classical measures, although trends are observed for higher activation during TMT-B. When corrected for processed items, higher activity for TMT-B in comparison to TMT-A is found within the SPL. The results are discussed in light of recent research designs, and simple to use correction methods are suggested.

Keywords: functional near-infrared spectroscopy; trail making test; processing speed; task complexity.

2.1.1. Introduction

Functional near-infrared spectroscopy (fNIRS) is an optical imaging method that is based on the physical properties of light in the near-infrared (NIR)-spectrum. Specifically, the method is based on the principle that light in the NIR-spectrum is capable of penetrating biological tissues, such as skin and skull, and is absorbed to different degrees depending on thickness, density, and optical properties of the tissue. Due to these properties, it is possible to measure relative changes in oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin in the human brain by placing fNIRS-sender and receiver optodes on a subject's head. The penetration depth of the NIR-light is about 2 to 3 cm (Haeussinger et al., 2014; Haeussinger et al., 2011). Compared with other imaging methods, fNIRS has some important advantages (Ehlis et al., 2014). The method is relatively economic, comparably easy to use, relatively insensitive to movement artifacts, has a high time resolution, and can be used in mobile applications. Therefore, fNIRS has been used extensively in environments and subject populations, where other neuroimaging methods could not be implemented. For example, using fNIRS, it is possible to measure cortical activation while subjects perform job interviews in ecologically valid environments as in the Trier social stress test (Rosenbaum, Hilsendegen, et al., 2018; Rosenbaum, Thomas, et al., 2018) or while they perform cognitive tasks, such as the verbal fluency test (VFT) (Heinzel et al., 2013; H. Zhang et al., 2015) or the trail making test (TMT) (Hagen et al., 2014; Muller et al., 2014; Nakahachi et al., 2010; Rosenbaum et al., 2016; Sawa et al., 2012; Shibuya-Tayoshi et al., 2007; Takeda et al., 2011).

While some of these tasks can be implemented within an event-related design, some are typically used with block designs. Importantly, using block designs in many cases preserves the ecological validity of a given task. For example, when subjects perform a free speech, it is ecologically valid to let them do so for the duration of a whole block instead of demanding single (and often simple) reactions to certain events. Usually, within a block design, the task and respective control conditions are performed for a certain duration of time (e.g., 30 to 40 s), and hemodynamic responses are averaged over this time period. In this way, fNIRS has been used to assess cortical hemodynamic changes during the

performance of different versions of the TMT. The TMT is a neuropsychological test with two parts: TMT-A and TMT-B. During TMT-A, subjects are asked to connect numbers written in circles in ascending numerical order (1-2-3…) while during TMT-B, subjects are asked to connect alternating encircled numbers and letters in numerical and alphabetical order (1-A-2-B…). The TMT-A assesses visuospatial abilities and the speed of information processing, whereas the TMT-B additionally assesses the executive function of task switching. As the TMT-A is the easier task, it could be assumed that hemodynamic responses are higher during TMT-B - the more demanding task - in brain areas that are recruited by both tasks. However, past investigations on the subject did not always find this effect, especially when time-restricted versions were used (Hagen et al., 2014; Muller et al., 2014).

One explanation why differences between TMT-A and TMT-B are not always observed may lie within different factors that contribute to the hemodynamic response: speed and task complexity. From the perspective of a computational model, we assume that during a task block the hemodynamic response of a given subject is positively related to the number of finished items (processing speed) that have been performed. On the other hand, tasks that are more complex should recruit more brain areas as more cognitive functions are needed for task completion and—when controlled for processing speed—should result in higher hemodynamic responses within a given subject. Usually, during TMT-A, more items are finished than during TMT-B, but the latter is a far more complex task, so both effects (complexity versus processing speed) might cancel each other out in many regions of interest (ROIs). This also becomes plausible when considering typical experimental comparisons: when the TMT-A is used as a comparison condition for TMT-B, both tasks must be equal in their attributes except for the variable of interest. As during the TMT-A more items are processed than during TMT-B, a correction method needs to be implemented. Such a correction method—e.g., by dividing the activation during the block by the number of completed items— would result in a parameter that reflects the relative blood oxygenation per item, comparable to the average blood oxygenation during an

event-related design, in which hemodynamic responses are averaged over each processed item.

Evidence from the effects of task complexity and processed items also comes from other research areas. For example, in a recent study by Artemenko et al. (2018), higher activity within areas of the cognitive control network was observed during the computation of less complex mathematical equations in comparison to more complex mathematical equations. This inverse association of complexity and hemodynamic responses was no longer significant when “performed computation” was used as a covariate (Artemenko et al., 2018). However, with respect to complexity, more complex mathematical operations—such as the carry-over effect in mathematical operations—are known to induce higher frontal cortical activation than less demanding mathematical operations. This effect is considered to represent increased working memory load during carry-over computations (Verner et al., 2013). In line with this, hemodynamic responses are also positively associated with working memory load in n-back tasks (C. Li et al., 2005; Molteni et al., 2008).

To test the effects of task complexity and processing speed, we investigated the hemodynamic responses during a block design of the TMT-A and TMT-B in the frontal and parietal cortex in three different speed conditions: slow speed, medium speed, and fast speed. In detail, we investigated blood oxygenation in the dorsolateral prefrontal cortex (DLPFC), inferior frontal gyrus (IFG), and superior parietal lobule (SPL)/ somatosensory association cortex (SAC). We assumed to observe an increase in O_2Hb levels from slow- to fast-speed conditions and higher activity in the TMT-B than the TMT-A when controlled for processing speed. This hypothesis is based on two assumptions: the hemodynamic response within a certain subject within a block design is positively associated to the number of items being processed (first assumption) and the complexity of the task (second assumption).

2.1.2. Material and Methods

Participants

Twenty healthy subjects were recruited for this study. The ethics committee at the University Hospital and University of Tübingen approved this study. Further, all subjects gave written informed consent. Exclusion criteria were acute mental or physical illness, neurological disorders, and chronic or acute diseases that affect brain functioning, such as diabetes or kidney failure. Out of the 20 subjects, 12 participants were female; the average age was 27 years (SD= 6.21), with 17.9 (SD= 4.43) years of education. All subjects were right-handed.

Procedures

During the measurement, subjects sat on a comfortable chair with a wooden clipboard in front of them. The clipboard was fixated on the table with a steepness of ~70 deg to allow working on a sheet of paper in upright head position and without horizontal head movements to minimize pressure on the optode fibers and resulting movement artifacts. Before the measurement, subjects completed a questionnaire assessing their demographic data and received instructions for the experiment. Afterward, they completed a version of the TMT-A and TMT-B in a slow (TMT-A) and fast manner (TMT-B) to become familiar with the tests and different processing speeds. In this training phase, subjects were given as much time as they needed to complete the whole test. Each version of the TMT consisted of 40 items. Subjects were instructed to work in the slow condition slowly, but not in a manner that they slow down with effort (e.g., by performing in very slow motion), in the fast condition as fast as possible, and in the moderate condition in between the speed of the slow and fast condition. At the beginning of the experimental blocks, subjects were asked to close their eyes and a pencil was placed in their hand. Afterward, subjects were instructed about the condition that would follow (TMT-A/TMT-B in slow/moderate/fast speed) and a corresponding test form was placed on the clipboard. Then, subjects were asked to open their eyes and start immediately with a 25-s block of task performance followed by 30-s rest. Afterward, the subjects were asked to close their eyes and the instruction for the next condition was given. In total, 18 blocks were assessed

with three repetitions of each condition (TMT-A versus TMT-B in the conditions slow versus moderate versus fast) in a randomized order.

Functional Near-Infrared Spectroscopy

We used a continuous wave, multichannel near-infrared spectroscopy (NIRS) system (ETG-4000 Optical Topography System; Hitachi Medical Co., Japan) with a temporal resolution of 10 Hz. Data were recorded with a semiconductor laser and avalanche diodes at two wavelengths (695 ± 20 and 830 ± 20 nm) with 4.0 ± 0.2 mW for each wavelength at each optode. In this study, we used three probesets placed on an electrode cap: 2 frontal probesets (reference points F3 and F4 according to the international 10–20 system (Jasper, 1958)) with 9 optodes each and one parietal probeset (reference point Pz) with 15 optodes. The electrode caps were positioned to 10–20 system reference points Fpz and Cz for each subject to guarantee correct optode placement. The whole setup consisted of 46 channels, covering parts of the bilateral DLPFC, IFG, and the SAC. For a detailed description of the probesets, see Rosenbaum et al. (2018). Corresponding brain areas of each channel were extrapolated from reference points as in the work by Singh et al. (2005) and Tsuzuki et al. (2014) based on the Colin 27 template.

Raw data were exported as TBL directory from the NIRS machine and reconstructed with self-written MATLAB code. The changes in absorbed NIR-light were transformed into relative O_2Hb and HHb levels by means of a modified Beer–Lambert law. fNIRS preprocessing was performed with MATLAB R2017a (MathWorks Inc., Natick) and included the following steps: bandpass filtering (0.001 to 0.1 Hz) based on discrete cosine transform (DCT)-II and inverse DCT-II filters, correlation-based signal improvement according to Cui et al. (2010), interpolation of single high artifact-loaded channels by visual inspection, independent component analysis (ICA) based reduction of clenching artifacts, and a further low cutoff filtering at 0.01 Hz. Note that the TMT in some cases induces high arousal artifacts that cannot be corrected by the ICA procedure due to too frequent high-amplitude signals. In this dataset, eight subjects showed such artifacts. In these cases, the signal was corrected by a principal component

analysis (PCA) reduction of the first component (Brigadoi et al., 2014). Additionally, data of all subjects were corrected for global signal changes by a Gaussian PCA-based kernel filter (X. Zhang et al., 2016). Finally, we standardized each subject's fNIRS data by the standard deviation of the concatenated signal of all channels. The 25-s blocks for each condition were averaged with a 10-s baseline correction and a linear detrending. Data were analyzed for five ROIs: SPL, bilateral DLPFC, and IFG chosen based on previous fMRI and NIRS studies on the subject (Hagen et al., 2014; Jacobson et al., 2011; Rosenbaum et al., 2016). Brain maps were computed with self-written MATLAB routines. The MATLAB code of the analysis is available on request.

Data Analysis

Statistical analysis was performed with IBM SPSS Statistics Version 24. For behavioral (performance) and fNIRS data, repeated measurement analyses of variances (ANOVAs) with the within-subject factors complexity (TMT-A versus TMT-B) and speed (slow versus moderate versus fast) were performed. fNIRS data were analyzed separately for each of the five ROIs, with correction for multiple testing of post-hoc analysis by the procedure of Armitage-Parmar (Sankoh et al., 1997). As post-hoc test, we used planned t-tests and linear contrast. We assumed to find an increase from slower to faster processing and higher hemodynamic responses during TMT-B in comparison to TMT-A. For effect sizes, Cohen's d and partial η^2 is reported. As we assumed that a correction for the number of performed computations would be needed to bring out the effects of task complexity, we performed two secondary analyses of the fNIRS data with corrected O_2Hb measures. To correct for performed computations, we used two different approaches. First, we regressed the average number of completed items per condition out of the hemodynamic response for each subject individually over all conditions and further analyzed residuals. Further, as the within-subject regression approach is not suitable for every NIRS investigation, we used an alternative more simple approach, in which we computed the ratio of a subject's given O_2Hb concentration and the average performance in the corresponding condition, resulting in the measure "O_2Hb per solved item" [(mmol

x mm) /item]. In this way, the O_2Hb values were set in relation to the individual number of processed items resulting in a metric comparable to the analysis of an event-related design, namely the relative blood oxygenation per solved item (during the block). Note that errors have not been used as covariates as error rates were very low (on average, below one error in five assessed blocks).

2.1.3. Results

Behavioral Data

With respect to behavioral performance in terms of completed items, a two (TMT) by three (speed) repeated measurement ANOVA showed significant main effects for TMT ($F_{(1,19)}$ = 23.19, p<.001, η^2=.55) and processing speed ($F_{(2,38)}$ = 93.41, p<.001, η^2=.83) as well as an interaction between both variables ($F_{(2,38)}$ = 34.84, p<.001, η^2=.67). Not surprisingly, the main effects indicated more processed items during TMT-A than TMT-B and a linear increase in processed items from slow- to fast-processing conditions. However, the interaction of TMT by processing speed indicated that the differences between TMT-A and TMT-B were higher in the fast-processing condition than in moderate and slow conditions (see Table 1). Indeed, post-hoc analysis of paired t-tests revealed only significant differences between TMT-A and TMT-B in the fast condition ($t_{(19)}$=7.49, p<.001, d=1.68). Correspondingly, with respect to speed, highest effect sizes were observed for TMT-A between moderate- and fast-speed conditions (TMT-A: $t_{(19)}$=10.63, p<.001, d=2.38; TMT-B: $t_{(19)}$=5.36, p<.001, d=1.2), followed by increases from slow to moderate speed (TMT-A: $t_{(19)}$=6.17, p<.001, d=1.38; TMT-B: $t_{(19)}$=6.5, p<.001, d=1.45).

With respect to error rates, we found a main effect for speed ($F_{(2,38)}$ = 10.46, p<.001, η^2=.35), which was driven by an increase in error rates from moderate- to fast-processing speed ($F_{(1,19)}$=9.11, p<.01, η^2=.32). However, average error rates were very low overall (below one per condition).

Table 1: Number of processed items and errors during TMT-A and TMT-B in the three speed conditions.

	TMT-A		TMT-B	
Variable	**Mean**	**SD**	**Mean**	**SD**
Processed items—slow	13.9	4.6	13.5	3.4
Processed items—moderate	19.2	5.7	17.8	5.2
Processed items—fast	30.1	5.8	22.5	5.8
Errors—slow	0.02	0.07	0.05	0.12
Errors—moderate	0.03	0.10	0.10	0.2
Errors—fast	0.20	0.31	0.23	0.27

Functional Near-Infrared Spectroscopy Data (mmol x mm)

As a first descriptive analysis, we checked the (uncorrected) activation of each channel against a zero mean distribution for each condition in multiple t-tests. Significant activations were found in each condition (see Table 2).

However, during the slow-processing condition, activations were mainly found within the SPL. Frontal channels were only significantly different from zero in the moderate- to high-speed conditions (see Table 2).

Table 2: Significant channels of the ROIs tested against zero in the experimental conditions. Note that p-values are not corrected for multiple tests, since the testing is used for descriptive purposes.

		TMT-A slow		TMT-B slow		TMT-A moderate		TMT-B moderate		TMT-A fast		TMT-B fast	
ROI	**Channel**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**
Left IFG	6	—	—	—	—	3.08	**0.006**	3.67	**0.002**	3.34	**0.003**	4.20	**0.000**
Left IFG	7	—	—	—	—	—	—	—	—	2.29	**0.034**	—	—
Left IFG	8	—	—	—	—	—	—	2.42	**0.026**	3.11	**0.006**	3.52	**0.002**
Left IFG	9	—	—	—	—	—	—	—	—	—	—	—	—
Left DLPFC	10	—	—	—	—	—	—	—	—	—	—	—	—
Left DLPFC	11	—	—	—	—	—	—	—	—	2.19	**0.041**	—	—
Left DLPFC	12	—	—	—	—	—	—	—	—	—	—	—	—
Right IFG	18	—	—	—	—	—	—	—	—	—	—	—	—
Right IFG	19	—	—	—	—	—	—	—	—	2.43	**0.025**	2.45	**0.024**
Right IFG	21	—	—	—	—	—	—	—	—	—	—	—	—
Right IFG	22	—	—	2.14	**0.045**	—	—	2.22	**0.039**	3.78	**0.001**	4.39	**0.000**
Right DLPFC	20	—	—	—	—	—	—	—	—	2.53	**0.020**	—	—
Right DLPFC	23	—	—	—	—	2.96	**0.008**	2.95	**0.008**	3.77	**0.001**	2.13	**0.046**
Right DLPFC	24	—	—	—	—	—	—	—	—	—	—	2.28	**0.034**
SAC	25	2.20	**0.040**	3.28	**0.004**	6.26	**0.000**	3.65	**0.002**	4.06	**0.001**	5.80	**0.000**
SAC	26	—	—	2.57	**0.019**	2.43	**0.025**	2.77	**0.012**	3.65	**0.002**	5.02	**0.000**
SAC	27	—	—	2.16	**0.043**	—	—	2.48	**0.023**	3.38	**0.003**	3.80	**0.001**
SAC	28	2.33	**0.031**	3.18	**0.005**	5.36	**0.000**	3.40	**0.003**	3.70	**0.002**	4.55	**0.000**
SAC	30	—	—	3.52	**0.002**	4.24	**0.000**	3.96	**0.001**	4.13	**0.001**	4.91	**0.000**
SAC	31	—	—	—	—	—	—	—	—	—	—	2.41	**0.026**
SAC	32	3.26	**0.004**	5.06	**0.000**	5.25	**0.000**	6.30	**0.000**	4.45	**0.000**	4.83	**0.000**
SAC	35	—	—	—	—	—	—	—	—	—	—	3.29	**0.004**
SAC	36	—	—	—	—	—	—	—	—	—	—	—	—

p-values are depicted in bold for better readability.

Within the analysis of the fNIRS data, we observed main effects for processing speed in the left IFG ($F_{(2,38)}$=4.98, p<.05, η^2=.21), left DLPFC ($F_{(2,38)}$=3.68, p<.05, η^2=.16), right IFG ($F_{(2,38)}$=7.25, p<.01, η^2=.28), and SPL ($F_{(2,38)}$=5.62, p<.01, η^2=.23) as indicated by a two (TMT) by three (speed) repeated measurement ANOVA. We found no significant interaction of TMT and processing speed or a significant main effect of TMT. As indicated by post-hoc analysis, all significant main effects of speed were characterized by linear increases in the hemodynamic response from slow to fast conditions: left IFG ($F_{(1,19)}$=10.32, p<.01, η^2=.35), left DLPFC ($F_{(1,19)}$=7.87, p<.01, η^2=.29), right IFG ($F_{(1,19)}$=11.54, p<.01, η^2=.37), SPL ($F_{(1,19)}$=11.38, p<.01, η^2=.37) (see Figs. 1 and 2).

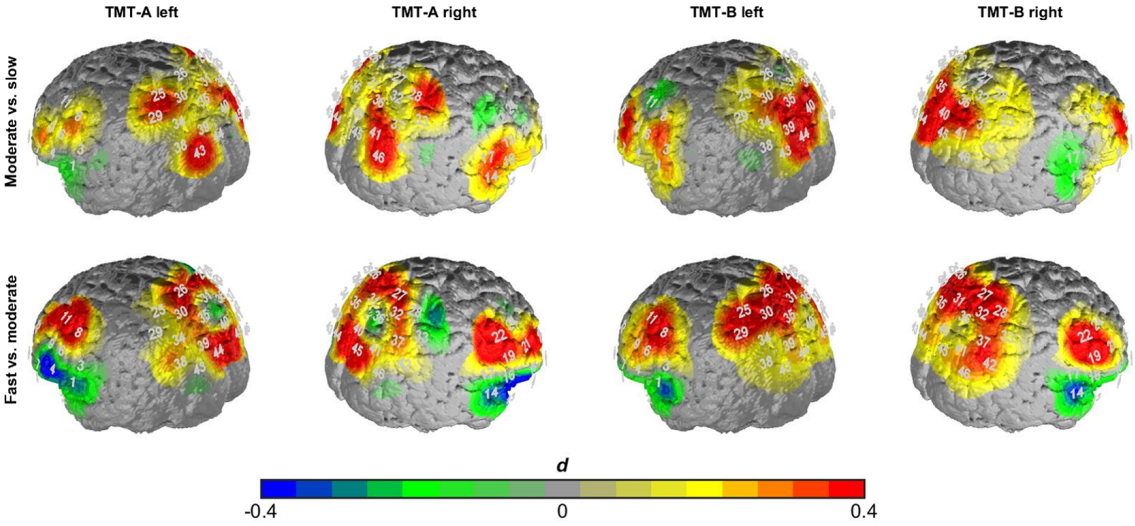

Figure 1: Contrast of the speed conditions in TMT-A and TMT-B. The upper row depicts the moderate-versus slow-speed contrast, and the lower row depicts the fast-versus moderate-speed contrast. Differences are shown in effect size Cohen's d.

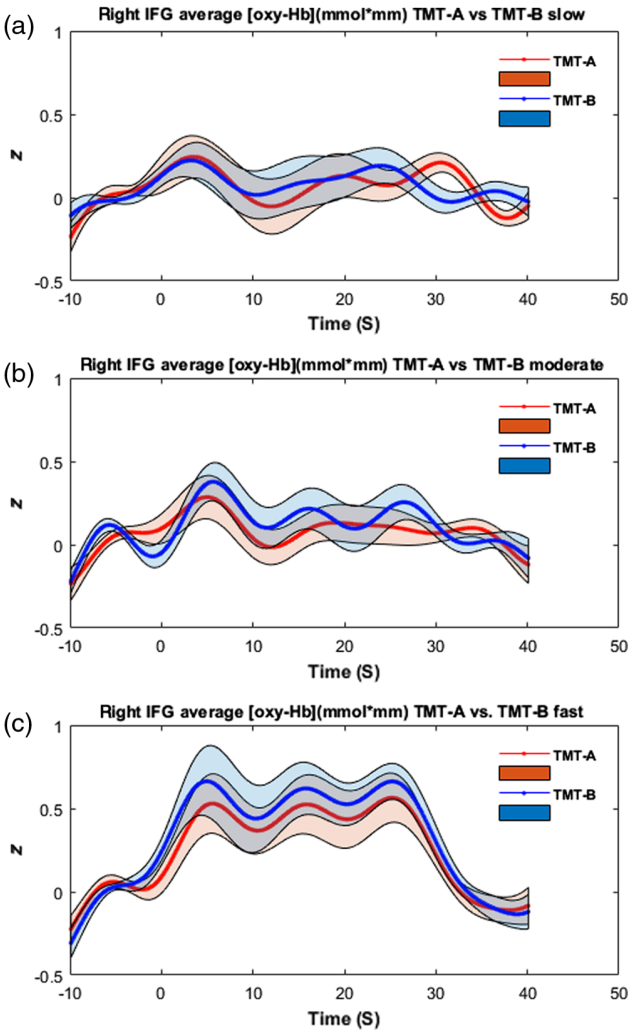

Figure 2: Hemodynamic responses during TMT-A (red) and TMT-B (blue) in the three speed conditions: (a) slow, (b) moderate, and (c) fast in the right inferior prefrontal cortex. Values are given in z-standardized scores of activation. Shaded areas indicate ±1 standard error of the mean.

Processing Speed Corrected Functional Near-Infrared Spectroscopy Data

Analyses of corrected fNIRS data revealed similar results between the regression and ratio approach. In both analyses, a comparable high main effect of TMT was observed in the SPL (regression-approach: $F_{(1,19)}=4.34$, $p<.05$, $\eta^2=.18$, ratio-approach: $F_{(1,19)}=4.41$, $p<.05$, $\eta^2=.19$), with higher hemodynamic responses during the TMT-B in comparison to TMT-A (see Figs. 3 and 4). Note that although only the main effect of TMT was significant, in Fig. 2, a descriptive trend can be observed for higher TMT-B versus TMT-A effects in the fast-speed condition than in moderate- and slow-speed conditions.

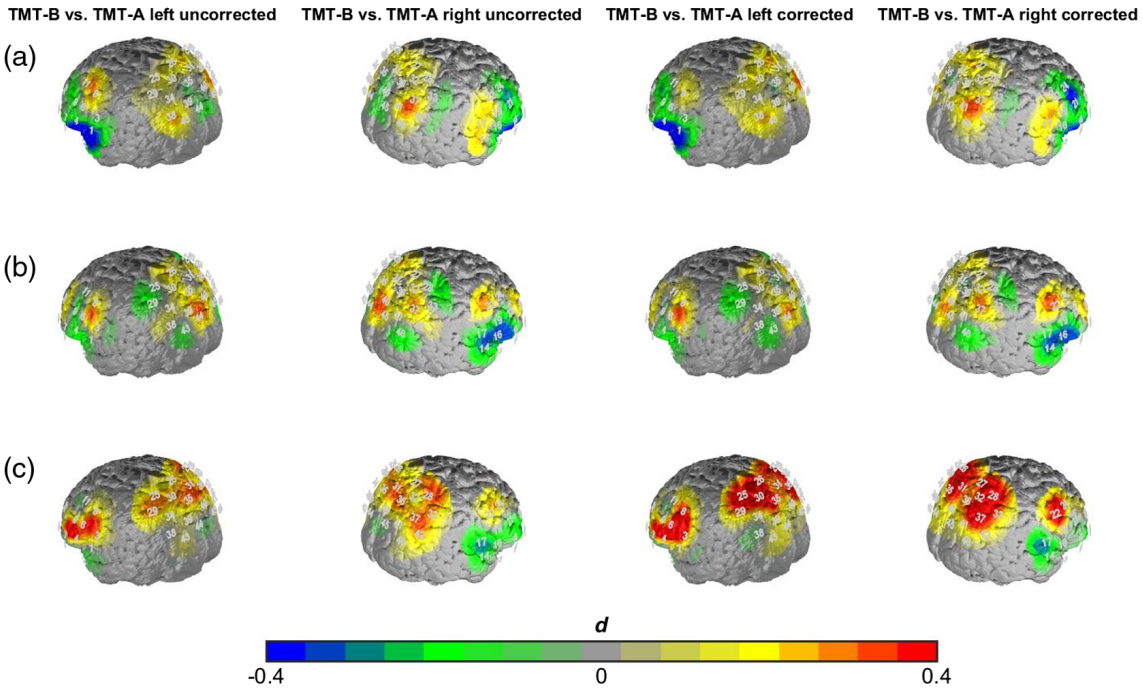

Figure 3: Contrast of the TMT-B versus TMT-A in the three speed conditions: (a) slow, (b) moderate, and (c) fast. The left columns depict the uncorrected O_2Hb measures and the right columns depict the ratio-corrected measures. Differences are shown in effect size Cohen's d.

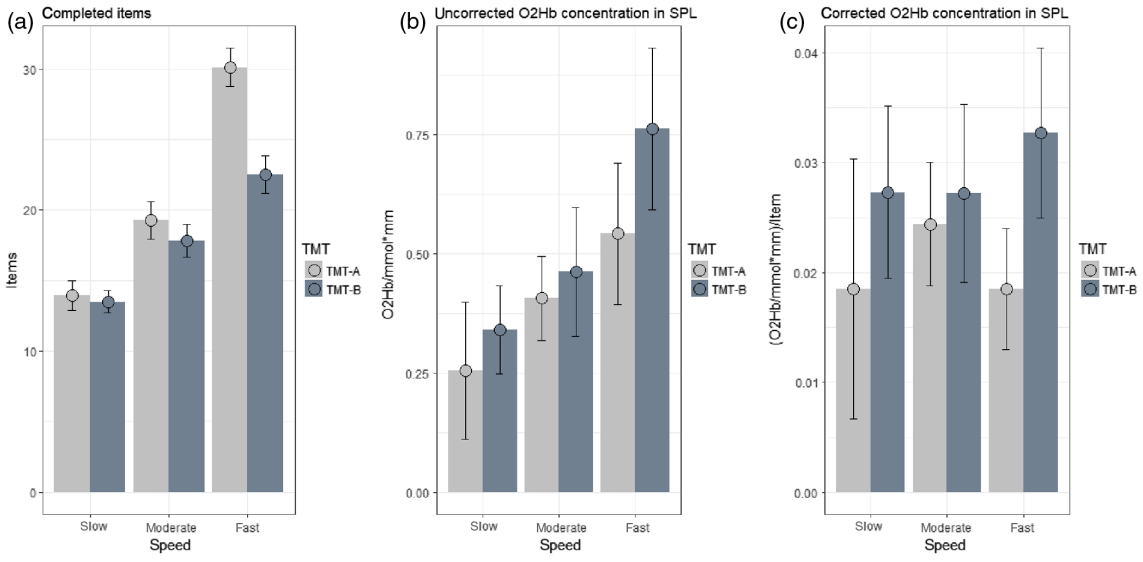

Figure 4: Effects of the speed condition and the TMT condition on (a) completed items, (b) uncorrected O_2Hb concentration in the SPL, and (c) corrected O_2Hb concentration in the SPL. Confidence intervals indicate 2 deviations of the standard error of the mean.

2.1.4. Discussion

This study aimed to investigate the effects of processing speed (slow versus moderate versus fast) and task complexity (TMT-A versus TMT-B) on hemodynamic changes as assessed with fNIRS in a block design. Our results showed that subjects were able to perform both tasks at the instructed speed levels, as indicated by a main effect of the factor processing speed. However, the usual behavioral effect of a reduced number of solved items during the TMT-B in comparison to TMT-A was only observed in the fast-speed condition. Notably, this effect would be assumed, as differences in task complexity should be observed when subjects perform as fast as possible. With respect to hemodynamic responses, we found high effects for the factor of speed in areas associated with cognitive control. Significant increases in the hemodynamic response were found in the bilateral IFG, the left DLPFC, and the SPL. Interestingly, no significant main effects were found for the factor of TMT, although trends were observed for higher activation during TMT-B. When controlled for the number of processed items by a regression or ratio approach, we observed increased activity during the TMT-B in comparison to TMT-A in the SPL.

Our results are in line with previous investigations that sought to investigate the effects of the TMT in time-controlled experiments (Hagen et al., 2014; Muller et al., 2014). In such experiments, usually no or only weak effects are found for the TMT contrast. From our results, we would assume that this is partly due to the lack of control for number of processed items. In the original neuropsychological measurement, the TMT is item-controlled, and time for test completion is used as a dependent variable. Adapted to neurophysiological measurements, an item-controlled version would need to use tailored windows for averaging to account for individually different working times between subjects. However, in some investigations, it may be desired to use a time-controlled version. Based on the results of this study, we would warn investigators that a time-controlled setting without accounting for the number of processed items might obscure the effects of the TMT contrast. In fact, in our investigation, the experimental effects of processing speed—as induced by different instructions—by far outnumbered the

differential effects of the TMT. This becomes especially important in investigations in elderly populations since elderly might compensate impairments during the TMT-B by further slowing down speed. In future investigations, it will be interesting to investigate in how far compensation leads to higher blood oxygenation per item. Furthermore, as during TMT-A more items are completed, higher hemodynamic responses might be present due to higher motor activity at least in some areas.

The experimental manipulation of processing speed led to high increases in activation within areas associated with cognitive control, namely bilateral IFG, left DLPFC, and SPL. Increased activity within these areas during the TMT is not surprising since the TMT is thought to involve cognitive functions associated with these areas and previous fNIRS investigations of the TMT also found activity here (Hagen et al., 2014; Muller et al., 2014; Nakahachi et al., 2010; Takeda et al., 2011). Both tasks— TMT-A and TMT-B—require visuospatial search function, motor speed, planning and attentional control. As depicted by the tests against zero, during slow-processing speed, only superior parietal areas showed significant hemodynamic responses as compared with baseline. As processing speed became faster in moderate and fast conditions, prefrontal areas became active. This effect might reflect the higher effort due to elevated processing speed. Indeed, this interpretation is in line with previous investigations of our group; higher activity was observed in the cognitive control network when subjects had to perform a math tasks under time pressure as compared with a task performance without time pressure (Rosenbaum, Hilsendegen, et al., 2018; Rosenbaum, Thomas, et al., 2018). Of note, during the typical TMT instruction, subjects are asked to work as fast as possible and as accurately as possible, which is comparable to our highspeed condition.

Interestingly, we observed significant differences between the TMT versions when the dependent variable was corrected for the number of processed items. In line with this, previous studies used different correction methods. For example, Sankoh et al. (1997) found significant effects between the TMT versions in an fMRI study, in which wait times were tailored to control for processed items. In our results, differences between TMT-A and TMT-B were only observed in the

SPL. Interestingly, lesion studies suggest that damage to the SPL is associated with impairments in tasks that involve the manipulation of material in working memory (Koenigs et al., 2009). The TMT-B, which requires task-switching and information updating in working memory during task completion, might be characterized by an increase in this cognitive function in comparison to TMT-A. Further evidence of the role of the SPL in task switching comes from functional MRI studies. For example, Gurd et al. (2002) observed in a task-switching version of the VFT that switching conditions were characterized by increases in SPL activity in comparison to nonswitching conditions.

In this study, we compared two different correction approaches to account for the number of processed items. Both approaches—a regression and ratio approach—yielded similar results. As most studies of the TMT do not include different speed conditions (which would allow for intrasubject correction by regression), we suggest using the simple ratio approach in such investigations as an additional dependent variable to check for differences when accounting for processing speed.

Despite these important results, some limitations and considerations have to be outlined. First, the fNIRS method only allows to measure the upper part of the cortex and spatial resolution is limited. Therefore, subcortical areas could not be measured and small areas of activation might have been missed. However, as the cognitive control network is in large parts located in cortical areas, fNIRS captures most of these areas. Second, although fNIRS is rather robust in terms of movement artifacts, the TMT induces movement and arousal artifacts. Especially, artifacts from teeth clenching, which is prominent in high arousal, induces high amplitudes of inverted U-shaped artifacts in the temporal muscles that have to be corrected before further processing of the data. If not, these artifacts might be mistakenly interpreted as strong hemodynamic responses. Therefore, we used strong correction methods by reducing data variance through deletion of PCA components and an additional PCA-based kernel filter. These correction methods however, might induce negative activation, which was not observed in this study. Third, we chose a design in which subjects were instructed to use self-imposed speed levels. From this instruction, one might argue that the

instruction itself might induce some kind of mental effort since subjects had to reduce their best performance speed willingly. However, we suggest that such mental effort should lead to higher activity. Since we observed reduced hemodynamic responses within the slower speed conditions, this factor might be negligible. In fact, we argue that the effort of the subjects increases as they had to perform faster. Although it is the aim of the used correction methods to correct for the influence of processed items, it is important to bear in mind that such a correction will also result—to some extend—in a correction for mental effort, as both constructs are associated. However, as long as subjects perform with equal effort in TMT-A and TMT-B versions (e.g., during fast speed—when performance is at the edge of skill), the correction methods will just result in a correction for processed items for the comparison of these two conditions.

Further, the study at hand aimed to investigate the effects of speed and complexity on the hemodynamic response in block designs. We only investigated a rather small sample size as we conducted a proof-of-principle study. As computed with G-Power (3.0), with the sample size of 20 subjects, effects up to $\eta^2=0.10$ were detectable between the TMT conditions ($\alpha=0.05$, $1 - \beta=0.80$, 2 measurements, $r= 0.50$). As we observed descriptive trends for higher hemodynamic responses during TMT-B as compared with TMT-A in the uncorrected data, it might be possible that we would have found such an effect without correction in a larger sample. However, it was not the intention of this investigation to raise any doubt on this point, but to show that the within-subject correction for completed items might yield a promising scale for the investigation of block-design hemodynamic data. It is important to bear in mind that the study at hand is no critique of block-design measurements per se, but a reminder that time-controlled settings may leave the potential confounder of performed items.

2.1.5. Conclusion

To the knowledge of the authors, this is the first study that investigated the effects of processing speed on activation within the cognitive control network during the TMT in a within-subject design. We observed high effects for processing speed that outnumbered the effect of the TMT contrast. In conclusion, sensitivity of the

TMT contrast might be increased by implementing some sort of correction method, as during TMT-A more items are processed than during TMT-B in time-controlled block designs. The suggested methods in this article might just be a first step in search of an optimal correction, and future research might yield better approaches than the simple ratio-based correction. However, the proposed method is a simple to use correction method that might be used in any block design investigation and paradigm, in which experimental conditions differ with respect to the number of processed items.

2.2. Study 2 – Age-related deterioration of performance and increase of cortex activity comparing time- versus item-controlled fNIRS measurement

The study was approved by the Ethics Committee of the University of Tuebingen (90/2009BO2).

The contents of this chapter are published:

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Keywords: functional near-infrared spectroscopy; trail making test; age, processing speed; cortex activity

Abstract

In our aging society, research into neurodegenerative processes is of great interest. Thereby, cortical activation under different neurocognitive conditions is considered to be a promising predictor. Against this background, the executive functions of a total of 250 healthy older adults (53-84 years) have been investigated using the Trail Making Test (TMT) and functional near-infrared spectroscopy in a block design. We investigated effects of age on the performance and cortical blood oxygenation during the TMT. Since it is assumed that older people may compensate for cognitive deficits by slowing their processing speed, we additionally analyzed the cortical blood oxygenation per solved item. Our results showed a significant decrease in processing speed in older participants compared to middle-aged individuals, however, also lower error rates during TMT part A. On a neurophysiological level, we observed increased cortical blood oxygenation in the older participants when completing the TMT. Finally, with respect to the combined measurement (O_2Hb/item), no significantly higher hemodynamic cortical response per item was found within the older participants. The results confirm a deterioration of cognitive performance and an increase of cortical activity with increasing age. The findings are discussed in the light of current research.

2.2.1. Introduction

The demographic development in Europe tends towards an ageing population due to an increasing life expectancy, caused by more prosperity, better medical care and a decline in the birth rate. Over the next two decades, the current number of people over 65 years of age is expected to double (Rees et al., 2012). Age is regarded as the greatest risk factor for the development of neurodegenerative diseases such as Parkinson´s disease (PD) and Alzheimer´s disease (AD). Currently, the worldwide prevalence of dementia is around 46 million. This is expected to rise to 132 million by 2050 (Alzheimer's Disease International, 2019), but the underlying mechanisms are still not sufficiently researched.

The brain, which is the affected organ in neurodegenerative diseases, is subject to lifelong structural (Fjell & Walhovd, 2010) and functional change (Hagen et al., 2014). Age-dependent processes of brain structure seem to make tissue more susceptible to neurodegenerative diseases (Fjell et al., 2014). In MRI studies, an age-dependent decrease in grey and white matter of 0.5-1% per year was observed (Courchesne et al., 2000; Good et al., 2001; Pfefferbaum et al., 1994; Walhovd et al., 2005). This phenomenon can be explained by a decrease in neuronal volume and synaptic branching (Fjell & Walhovd, 2010). Affected are mainly the prefrontal cortex (PFC) and hippocampus brain regions, which play an essential role in learning and memory storage (Morrison & Baxter, 2012). The decline in cognitive ability in old age primarily affects working and episodic memory, resulting in reduced processing speed and decreased mental flexibility (Hedden & Yoon, 2006). In addition, deficits in decision making and speech processing are mentioned (Glisky, 2007). Against this background, it is essential to be able to detect neurodegenerative processes that lead to a decline in cognitive function at an early stage.

Functional near-infrared spectroscopy (fNIRS), a neurophysiological measurement technique that is based on the detection of hemodynamic changes in cortical oxygenated and deoxygenated blood levels that follow neuronal activity (Colier et al., 1995), combines decisive advantages, such as investigations under ecologically valid conditions (e. g. tests in realistic environment instead of a

narrow tube (MRI), social interaction, investigations in an upright sitting position) and insensitivity to movement artifacts (Ehlis et al., 2014).

In this study, the advantages of fNIRS were used to measure the cognitive abilities of a large group of participants under realistic and physiological conditions using the Trail Making Test (TMT). The TMT is used to assess cognitive performance. It facilitates the assessment of various neuropsychological parameters such as mental flexibility, working memory, visuomotor processing speed and executive functions (Arbuthnott & Frank, 2000). Classically, the TMT is divided into parts A and B, but there is also part C in some versions of the TMT. In the TMT-A, the subject is asked to connect numbers on a sheet of paper in ascending order with a pencil as quickly as possible. Both TMT versions (A and B) require visual search function, motor speed abilities, and recall of working memory. The TMT-B additionally assesses the ability of task-switching by requiring participants to alternately connect numbers and letters in ascending order of the number chain / alphabet (Stuss & Levine, 2002). The TMT is usually evaluated by measuring the time to completion (Spreen & Strauss, 1998). If the respondent makes a mistake, he or she is immediately told that he or she made a mistake and pointed back to the last correct item. The time measurement is not interrupted during this time (Tischler & Petermann, 2010). TMT-A is considered a simpler task. For this reason, higher activation during TMT-B can be expected in brain regions recruited for both tasks. Contrary to these expectations, this effect has not been reported consistently in the literature, especially when block designs are used (Hagen et al., 2014). One possible explanation is that both, processing speed and task complexity, play a role in the level of blood oxygenation (Arbuthnott & Frank, 2000; Rosenbaum, Blum, et al., 2018).

In one of our previous studies, we found that task complexity and processing speed both have effects on cortical blood oxygenation, implicating that results from TMT paradigms in older individuals might be compromised if the participants slow down speed to compensate for age-related deficits (Rosenbaum, Blum, et al., 2018). Therefore, we suggested to either use a TMT paradigm in which different speed levels are realized or to use an additional dependent variable:

blood oxygenation per solved item. The correction method, developed in our first study, relates averaged performance and individual blood oxygenation per item (relative O_2Hb values per processed item within the block ((mmol x mm)/item). The validity of this simplified correction method was tested by a parallel regression calculation with analysis of the participants' individual O_2Hb values after regressing out the number of processed items for all TMT conditions in three different speed levels.

Regarding behavioral effects, the comparison with previous literature confirmed a deterioration in performance during the TMT with increasing age (Mitrushina et al., 2005; Tombaugh, 2004), with both an increase in processing time (Hamdan & Hamdan, 2009; Rasmusson et al., 1998) and a decrease in accuracy (Bäckman et al., 2004; Tombaugh, 2004). A study by Rodewald et al. (2012) compared the TMT processing times of different age groups and found an age effect, characterized by reduced processing speed for the TMT-B in older participants. A longitudinal study by Rasmusson et al. (1998) revealed similar results confirming increased completion times for both TMT-A and TMT-B for older participants. While the processing time for TMT-A remained unchanged, a significant increase for the TMT-B time within the two-year period was found.

On a neurophysiological level, various studies agree that older adults show higher cortical activation than younger adults when performing demanding tasks (Cabeza et al., 2002; Cabeza et al., 2004; Payer et al., 2006), which has often been interpreted as evidence of a compensatory mechanism. fNIRS studies, for example, by Herrmann et al. (2006) and Muller et al. (2014) detected altered activation patterns in older participants with more bilateral activation in the dorsolateral prefrontal cortex (DLPFC) as compared to younger individuals. Apart from the bilateral reorganization, a correlation between overactive brain regions and performance success was noticed in elderly (Rossi et al., 2004). A well-known model that incorporates the idea of a compensation mechanism is the Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) – model (Patricia A Reuter-Lorenz & Cappell, 2008). It assumes that older adults respond to increasing cognitive task severity with additional recruitment and activation of brain regions. Since older individuals reach their load limit earlier

than younger persons, an increase in brain activation in older individuals can already be expected in the lower/medium task load in the sense of a classical compensation. However, the additional possible recruitment is limited, which is why older adults in the higher performance range eventually show a worsened performance and lower activation compared to the younger group (Patricia A Reuter-Lorenz & Cappell, 2008). Another frequently discussed hypothesis to the age-related decline in cognitive performance is the neural dedifferentiation (Goh, 2011; S.-C. Li et al., 2001; Payer et al., 2006). The hypothesis implies a decrease in neural differentiation with age, accompanied by lower selectivity in neural processing and functional specificity of individual brain regions (S.-C. Li et al., 2001; S. C. Li & Rieckmann, 2014).

In light of the aforementioned cognitive aging models, our study aims to contribute to a better understanding of the interaction of neural activations and performance in older age by comparing two different approaches: First, the cortical blood oxygenation (O_2Hb during task completion; time-controlled) and second, as an additional index, the item-controlled blood oxygenation (O_2Hb/item). In addition, we investigated the effects of age on behavioral performance during the TMT (solved items). We assumed an age-related reduction of processing speed, as well as an increased hemodynamic response during the TMT. Furthermore, by comparing the classical time-corrected results with the results of a within-subject correction method for the number of processed items, we expected to highlight influences of performance on brain activation.

2.2.2. Material and Methods

Study design

Data have been collected during the TREND-study at the Department of Psychiatry and Psychotherapy of the University Hospital of Tuebingen, Germany which started in 2009. Since then, a total of 1201 volunteers have been examined every 2 years. The aim of the TREND study is to investigate possible prodromal markers for neurodegenerative diseases. After a detailed anamnesis, the participants complete a circle of test stations with several neurological individual examinations. As part of the Consortium to Establish a Registry for Alzheimer`s

Disease (CERAD) Plus test battery, brain activity was measured by functional near-infrared spectroscopy (fNIRS) while the participants completed the Trail Making Test (TMT). The cohort is composed of 250 older adults, covering an age range from 53 to 84 years. The available data were collected during the second follow-up in spring 2013 - autumn 2014 and represent a cross-sectional study. The study design was reviewed and approved by the Ethics Committee at the University Hospital and University of Tuebingen and complies with the standards of the declaration of Helsinki in its latest version. Autonomous informed consent was obtained from all participants.

Participants

In the current study we extracted a sample of 125 older participants (>66 years old) and matched a group of 125 younger participants (<66 years old) according to gender and neurodegenerative pre-existing conditions. The younger group showed an average age of 59.94 (*SD* = 3.60) years and 14.43 (*SD* = 2.52) years of education. Within the older cohort the mean age was 72.27 (*SD* = 4.58) years with an average of 14.24 (*SD* = 2.78) years of education. The groups did not differ in terms of gender distribution ($\chi^2(1) = 0.41$, $p = .520$) or years of education ($t(248) = 0.57$, $p = .568$, d = .072) and neurodegenerative pre-existing conditions (amnestic Mild Cognitive Impairment (aMCI) ($\chi^2(1) = 1.36$, $p = .243$); REM sleep behavior disorder (RBD) ($\chi^2(1) = .03$, $p = .860$).

Within the whole sample of 250 participants, 86% took medication: Mainly blood pressure medication (42%), anticoagulants (26%) and antidepressants (10%). The age groups differed concerning the blood pressure medication ($\chi^2(1) = 912.91$, $p = .000$) (53% within the older group, 30% within the younger group took blood pressure medication) and anticoagulants/antiplatelet drugs ($\chi^2(1) = 26.68$, $p = .000$) (41% within the older group, 12% within the younger group took anticoagulants/antiplatelets). Concerning the antidepressants ($\chi^2(1) = 1.659$, $p = .198$) (7% within the older group, 12% within the younger group took antidepressants) no differences were detectable.

The medication was recorded independently of the underlying diagnosis, since an influence of medication on the fNIRS signal cannot be excluded.

Experimental setup

1. Trail Making Test

The measurement was performed in a quiet darkened room in order to reduce the influence of light or auditory stimuli on the measurement results. The participants were instructed to take an upright sitting position and to avoid head movements as far as possible, since a displacement of the optodes and movement artifacts should be prevented. A DIN A4 worksheet was attached to an inclined desk to ensure physiological operation. After attaching the fNIRS-optodes, the participants received a detailed working instruction and a pencil. They were instructed to complete the test as quickly as possible „without lifting the pen“ as described in the CERAD_Plus test battery. Each version of the TMT consists of 25 items.

The procedure started with a five-minute resting-state measurement with closed eyes. Then the TMT started in the order TMT-C, TMT-A, TMT-B, which was repeated once. Each TMT measurement began with a 10 second baseline measurement with open eyes; afterwards the TMT was performed for 30 seconds and then stopped. TMT-C was used as a modification of the usual TMT to assess cortical activation solely related to motor skills by requiring the participants to trace dotted lines (control condition). The number of processed items and errors was documented.

2. Functional Near-Infrared Spectroscopy

In the current study, brain activation was detected by a multichannel fNIRS system (ETG-4000 Optical Topography System; Hitachi Medical Co., Japan). Multiple optodes of two wavelengths (695 nm and 830 nm) measure oxy- and deoxyhemoglobin simultaneously with a sampling rate of 10 Hz.

Three probesets (1A, 1B, 2) with 38 channels were fixed on the volunteer’s head using an optode holder cap: One parietal (14 channels) and two covering left and right fronto-temporal areas (12 channels each; see Figure 5. The exact placement of the optode holder cap on the skull was controlled by the reference points Fpz and Cz according to the international 10-20 system. The arrangement

of the channels to corresponding brain regions was based on the Colin template (Singh et al., 2005; Tsuzuki & Dan, 2014; Tsuzuki et al., 2007).

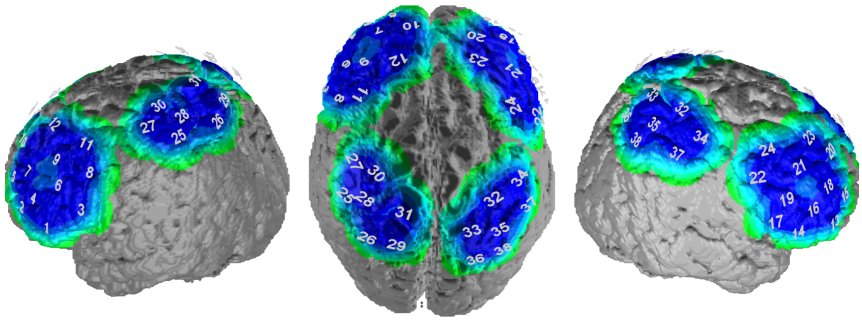

Figure 5: Probeset coordinates

Data-Analysis

The measured fNIRS signals were processed and visualized with the ETG-4000-system- software. The data set was then exported (CSV-file format) and analyzed with MATLAB R2017a (MathWorks Inc., Natick, USA).

Data on cortical blood oxygenation in terms of oxygenated (O_2Hb) and deoxygenated hemoglobin (HHb) were computed by means of a modified Beer-Lambert-Law. Preprocessing included correction of high amplitude artifacts by the TDDR correction (Fishburn et al., 2019), bandpass filtering (0.01-0.1 Hz), and a correlation based signal improvement described by Cui et al. (2010). The following steps included a visual inspection of the data and interpolation of strongly deviating outlier channels by proximate channels as well as a correction for the global signal changes by a PCA-based kernel filter (X. Zhang et al., 2016). Furthermore, data were individually averaged across block repetitions with a baseline correction to eliminate baseline drifts. Additionally to O_2Hb levels, we computed an item-controlled within-subject correction by dividing the fNIRS O_2Hb values by the number of completed items, generating the additional variable: O_2Hb/processed items ((mmol x mm)/item) (Rosenbaum, Blum, et al., 2018).

After pre-processing, fNIRS data were further analyzed with IBM SPSS Statistics Version 24. The analysis of behavioral data was calculated by a two (age: younger vs. older participants) by three (TMT: TMT-C vs. TMT-A. vs. TMT-B)

repeated measures ANOVA. For post-hoc analysis Helmert contrasts (TMT-C versus TMT-A/B, TMT-A versus TMT-B) were used. To account for the missing normal distribution, number of errors committed during the TMT was compared between the two age groups by using non-parametric Mann-Whitney tests. To analyze fNIRS data, we performed repeated measurement MANOVAS with the factors age (<66 and ≥66; between-subjects) and TMT (levels A, B, C; within-subjects), for the depended variables of each ROI (left and right dorsolateral prefrontal cortex (left DLPFC, right DLPFC), left and right inferior frontal gyrus (left IFG, right IFG), left and right somatosensory association cortex (left SAC, right SAC). In case of significant effects in the MANOVA, we investigated univariate statistics and corrected for multiple comparisons by the Benjamini-Hochberg procedure. Further, we repeated the analysis with item-corrected data. Finally, we explored on how far the association between the factor of age and the dependent variables (fNIRS data, completed items) is linear or quadratic. For this aim, we computed mixed level models in which we regressed age and the quadratic age term in separate models with random intercepts on the dependent variables (items, left DLPFC, right DLPFC, left IFG, right IFG, left SAC, right SAC).

2.2.3. Results

Behavioral performance

On a behavioral level, a two (age: younger vs. older participants) by three (TMT: TMT-C vs. TMT-A. vs. TMT-B) ANOVA showed a significant main effect of TMT ($F(1.95, 477.99) = 1536.91$, $p = .000$, partial $\eta^2 = 0.86$) and a main effect of age ($F(1, 245) = 37.64$, $p = .000$, partial $\eta^2 = 0.13$). Post hoc analysis revealed fewer processed items during TMT-A/TMT-B in comparison to TMT-C ($F(1, 245) = 1568.75$, $p = .000$, partial $\eta^2 = 0.87$), fewer processed items during TMT-B in comparison to TMT-A ($F(1, 245) = 1511.33$, $p = .000$, partial $\eta^2 = 0.86$) and fewer processed items in older than in younger participants (TMT-A: $t(211.73) = 6.13$, $p = .000$, d = 0.78), (TMT-B: $t(240.33) = 4.45$, $p = .000$, d = 0.56) (table 3). Male and female participants did not differ in terms of the number of processed items

(TMT-A: $t(248) = 4.16$, $p = .677$, d = .05), (TMT-B: $t(248) = 1.41$, $p = .161$, d = 0.18).

In addition, we found an interaction of age by TMT ($F(1.95, 477.99) = 16.22$, $p = .000$, partial $\eta^2 = .06$) reflecting that the age groups showed significant differences during TMT-A/B in comparison to TMT-C ($F(1, 245) = 30.45$, $p = .000$, partial $\eta^2 = 0.11$) as well as for the contrast of TMT-A and TMT-B ($F(1, 245) = 4.80$, $p = .029$, partial $\eta^2 = .02$). As indicated by these results, age and number of processed items were negatively correlated (TMT-A: $r(248) = - 0.50$, $p = .000$), (TMT-B: $r(248) = - 0.40$, $p = .000$).

With respect to error rates, we found a significantly reduced error rate in the older participants (mean = .08 errors ($SD = .21$) in comparison to the younger participants (mean = .19 errors ($SD = .31$) during TMT-A ($U = 6780.50$, $z = - 2.63$, $p = .008$, $r = - .17$) (table 3). However, groups did not differ in error rates during TMT-B and TMT-C. A within-subject analysis of the older participants found no correlation between the number of completed items and the number of committed errors for TMT-A: $r(125) = - 0.04$, $p = .628$). Furthermore, there were no differences in error rates between men and women (TMT-A: ($U = 6933.00$, $z = - 1.93$, $p = .053$, $r = - .12$), (TMT-B: $U = 7213.00$, $z = - 1.11$, $p = .265$, $r = - .07$). In total, error rates were very low (TMT-C = .02, mean TMT-A = .12, TMT-B =.19) and most participants performed without errors at all (TMT-C = 97%, TMT-A = 81%, TMT-B = 76%). For this reason, we did not include error rates in the evaluation of item-corrected NIRS data.

Table 3: Number of processed items and errors during TMT-A, TMT-B and TMT-C, depending on age

		Middle-aged participants (<66 years)		Older participants (>66 years)	
		mean	*SD*	mean	*SD*
TMT-A	processed items	22.31	3.14	19.14	4.87
TMT-B	processed items	11.50	4.09	9.28	3.41
TMT-C	processed items	23.86	0.85	23.57	1.49
TMT-A	mean error rates	0.17	0.31	0.08	0.21
TMT-B	mean error rates	0.22	0.49	0.16	0.36
TMT-C	mean error rates	0.01	0.06	0.03	0.13

fNIRS Time-corrected data

Not surprisingly, a repeated measures MANOVA determined that mean blood oxygenation levels showed a statistically significant difference between the different TMT conditions (TMT-A, TMT-B, TMT-C) ($F(12, 237) = 2.51$, Wilk's $\Lambda = 0.89$, $p = .004$, partial $\eta^2 = 0.11$). Further, we observed a main effect for age (younger vs. older participants) ($F(6, 243) = 2.69$, Wilk's $\Lambda = 0.94$, $p = .015$, partial $\eta^2 = .06$).

The univariate comparison of the main effect for TMT showed significantly deviating O_2Hb levels in left IFG ($F(2, 496) = 7.02$, $p = .001$, partial $\eta^2 = .028$) and right IFG ($F(1.94, 482.23) = 4.46$, $p = .013$, partial $\eta^2 = .02$) and tendencies in the right DLPFC ($F(1.79, 443.14) = 3.76$, $p = .028$, partial $\eta^2 = .02$) and left DLPFC ($F(1.92,475.26) = 3.35$, $p = .038$, partial $\eta^2 = .01$). Post-hoc analysis revealed differences between TMT-C contrasted to TMT-A/B in left and right IFG and in left and right DLPFC: left IFG: ($F(1, 248) = 12.94$, $p = .000$, partial $\eta^2 = .05$); right IFG: ($F(1, 248) = 8.80$, $p = .003$, partial $\eta^2 = .03$), left DLPFC ($F(1, 248) = 5.92$, $p = .016$, partial $\eta^2 = .02$), right DLPFC ($F(1, 248) = 7.02$, $p = .009$, partial $\eta^2 = .03$) (figure 6). No differences were observed between TMT-A and TMT-B (all $p>.05$). The univariate analysis of the main effect of age specified significant effects in left DLPFC ($F(1, 248) = 8.77$, $p = .003$, partial $\eta^2 = .03$), right SAC ($F(1, 248) = 10.00$, $p = .002$, partial $\eta^2 = .04$) and tendencies in the left SAC ($F(1, 248) = 4.99$, $p = .026$, partial $\eta^2 = .02$) and was characterized by higher O_2Hb levels in older as compared to younger participants (figure 7).

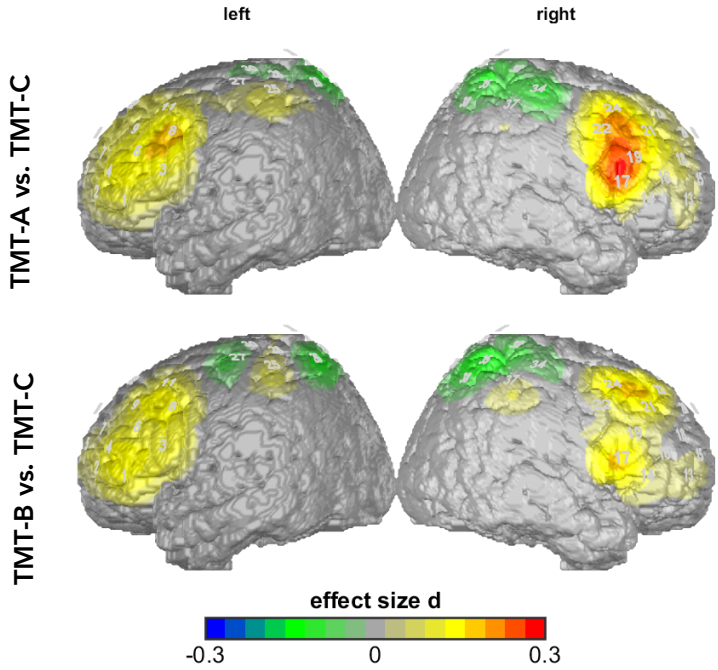

Figure 6: Time-corrected data (uncorrected O_2Hb values): Activity in TMT-A and TMT-B versus TMT-C. Higher activity in TMT-A and TMT-B compared to TMT-C. Differences are shown in effect size Cohen's d.

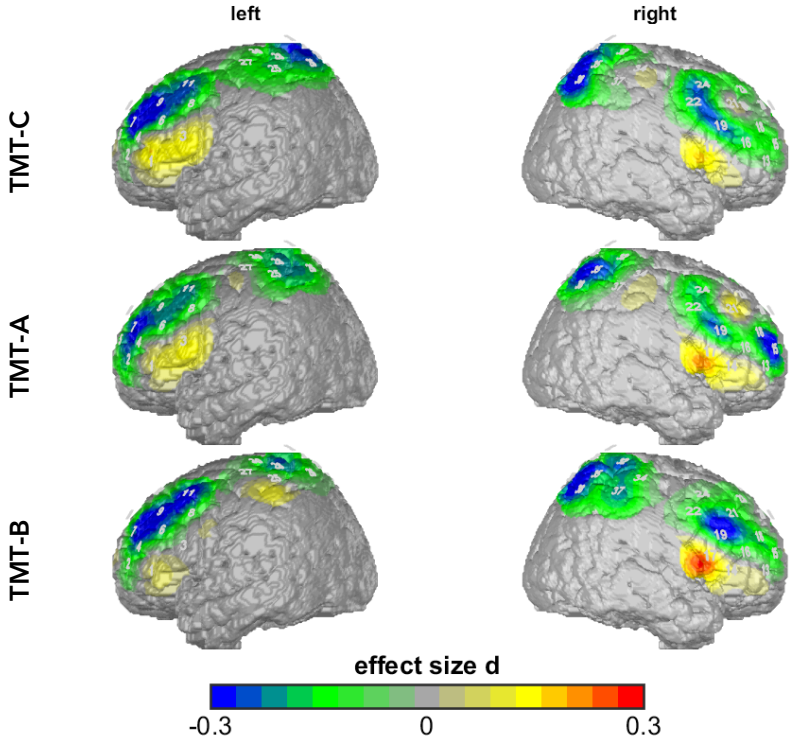

Figure 7: Time-corrected data (uncorrected O_2Hb values): Age effects (participants age < 66 vs. participants age ≥ 66) in TMT-A, TMT-B and TMT-C. Older participants showed higher activity compared to younger participants. Differences are shown in effect size Cohen's d.

fNIRS Item-corrected data

When data were corrected for the number of processed items, 13 multivariate outliers were found, as assessed by the Mahalanobis distance ($p > .001$) and excluded from the analysis. 30.8% of the 13 excluded participants belonged to the younger group and 69.2% to the older group, the sex ratio was 46.2% male and 53.8% female.

As with the time-corrected data, a significant main effect for TMT ($F(12, 224) = 6.07$, Wilk's $\Lambda = 0.76$, $p = .000$, partial $\eta^2 = 0.25$) was observed in the item-corrected analysis.

The main effect for TMT in the univariate analysis showed up in four ROIs: left IFG ($F(1.34, 314.48) = 36.40$, $p = .000$, partial $\eta^2 = 0.13$), right IFG ($F(1.28, 301.23) = 6.74$, $p = .006$, partial $\eta^2 = .03$), left DLPFC ($F(1.18, 277.91) = 8.15$, $p = .003$, partial $\eta^2 = .03$), right DLPFC ($F(1.18, 277.35) = 10.47$, $p = .001$, partial $\eta^2 = .04$) (figure 8). Post-hoc analysis of item-corrected data indicated significant differences between TMT conditions for the first Helmert contrast (TMT-C vs. TMT-A/B) as well as for the second Helmert contrast (TMT-A vs. TMT-B). With respect to the first Helmert contrast we observed differences in four ROIs: lDLPFC: ($F(1, 235) = 14.23$, $p = .000$, partial $\eta^2 = .06$); rDLPFC: ($F(1, 235) = 18.59$, $p = .000$, partial $\eta^2 = .07$); lIFG: ($F(1, 235) = 52.10$, $p = .000$, partial $\eta^2 = 0.18$) and rIFG: ($F(1, 235) = 17.56$, $p = .000$, partial $\eta^2 = .07$). Further, through the item-correction, effects for the second Helmert contrast (TMT-A vs. TMT-B) became also visible: left IFG: ($F(1, 235) = 28.59$, $p = .000$, partial $\eta^2 = .11$) and right DLPFC ($F(1, 235) = 6.13$, $p = .014$, partial $\eta^2 = .03$). The effect indicates higher cortical blood oxygenation per item during TMT-B in comparison to TMT-A.

No between-subjects effect for age was seen in the item-corrected data ($F(6, 230) = 1.41$, Wilk's $\Lambda = 0.97$, $p = .212$, partial $\eta^2 = .04$).

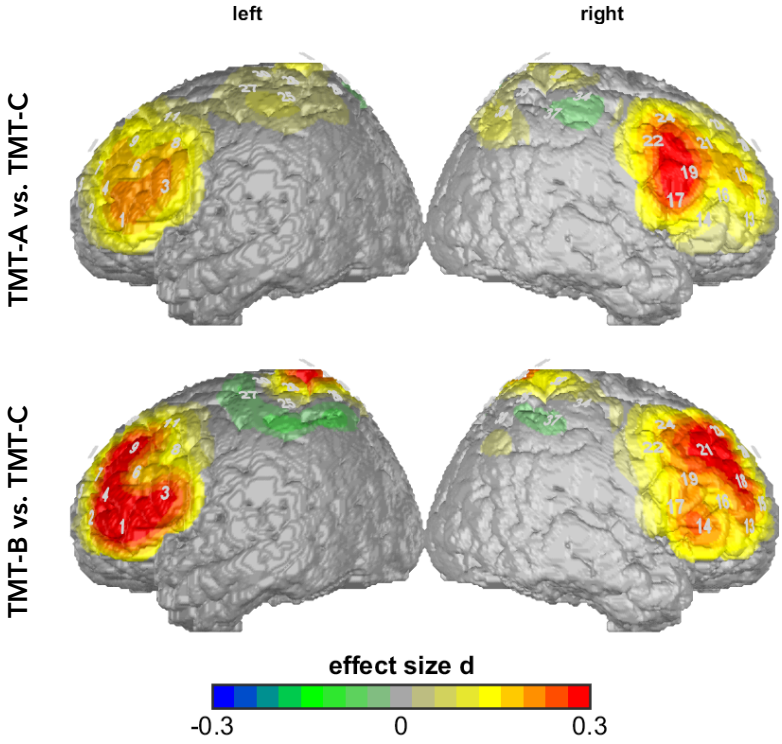

Figure 8: Item-corrected data (O_2Hb/item): Activity in TMT-A and TMT-B versus TMT-C. Differences are shown in effect size Cohen's d.

Exploratory Analysis

The results of our item corrected analysis raised the question of why the effect of age was no longer significant, as older individuals generally showed higher brain activity and fewer completed items. Therefore, we explored the association of age, processed items and O_2Hb levels in greater detail.

Interestingly, while the analysis indicated a linear negative relationship between age and completed items, we observed a negative quadratic relationship between age and the left DLPFC and right SAC (table 4 and figure 9/10). This negative quadratic relationship showed a turning point between 65 to 70 years and was characterized by relative increases in O_2Hb levels up to 65 to 70 years and decreases in O_2Hb levels with increasing ages above 70 years.

Table 4: Results of the mixed models on the polynomic relationship between age and the DVs.

	Model 1	Model 2	
DV	age	age	age²
Items	$t_{(745)}$=14.065	$t_{(744)}$=.975	$t_{(744)}$=.324
	p<.001	p>.1	p>.1
	β=-.18		
left IFG	$t_{(248)}$=-.551	$t_{(247)}$=1.248	$t_{(247)}$=-1.282
	p>.1	p>.1	p>.1
right IFG	$t_{(248)}$=-.85	$t_{(247)}$=.143	$t_{(247)}$=-.191
	p>.1	p>.1	p>.1
left DLPFC	$t_{(247)}$=1.759	$t_{(247)}$=3.621	$t_{(247)}$=-3.524
	p<.1	**p<.001**	**p<.001**
	β=0.01	β=0.35	β=-0.003
right DLPFC	$t_{(248)}$=.654	$t_{(247)}$=1.917	$t_{(247)}$=-1.883
	p>.1	**p<.1**	**p<.1**
		β=0.17	β=-0.001
left SAC	$t_{(248)}$=1.717	$t_{(247)}$=1.886	$t_{(247)}$=-1.791
	p<.1	**p<.1**	**p<.1**
	β=0.008	β=0.09	β=-0.001
right SAC	$t_{(248)}$=3.263	$t_{(247)}$=2.162	$t_{(247)}$=-1.978
	p<.001	**p<.05**	**p<.05**
	β=0.016	β=0.183	β=-0.001

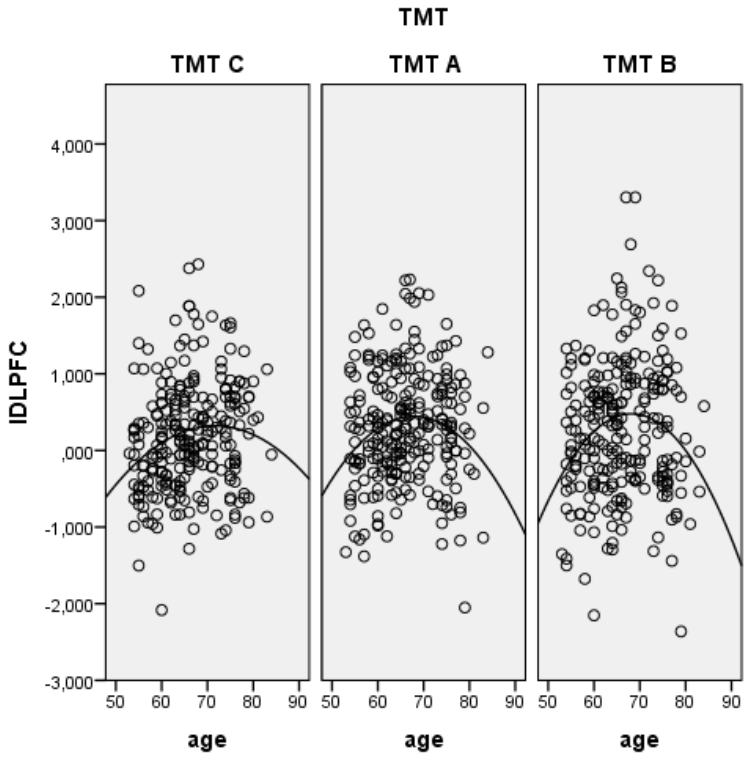

Figure 9: Negative quadratic relationship between age and fNIRS data in left DLPFC

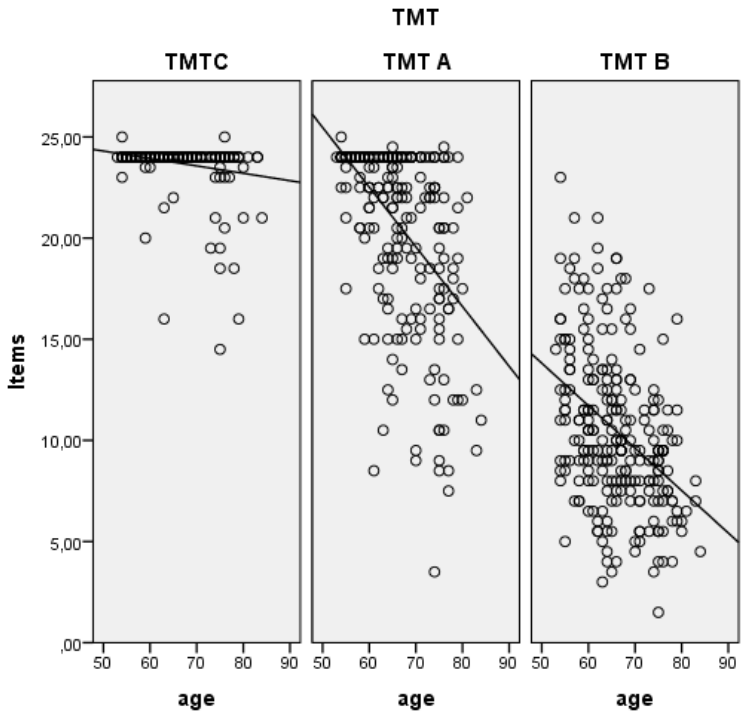

Figure 10: Negative linear relationship between age and number of completed items.

Controlling for confounders

To determine the influence of medication, we additionally calculated MANCOVAs with the factors blood pressure medication and anticoagulants/antiplatelet drugs as covariates. Interestingly, the results of the time-corrected data remained unchanged (differences in blood oxygenation between the three TMT conditions TMT-A, TMT-B, TMT-C ($F(12, 235) = 2.38$, Wilk's $\Lambda = 0.89$, $p = .006$, partial $\eta^2 = 0.11$)) and (differences in blood oxygenation between the two age groups ($F(6, 241) = 3.35$, Wilk's $\Lambda = 0.92$, $p = .003$, partial $\eta^2 = .08$)), whereas the item-corrected analysis showed an additional age effect between the groups (younger versus older participants ($F(6, 241) = 2.86$, Wilk's $\Lambda = 0.93$, $p = .01$, partial $\eta^2 = 0.07$)).

2.2.4. Discussion

The aim of the study was to investigate the effects of age on performance and cortical blood oxygenation during the completion of a cognitively demanding task. As aging is associated with compensatory developmental effects, we investigated the use of a within-subject correction for the number of completed items to determine an influence of performance on the activity pattern in the TMT in order to accentuate age-related changes.

With respect to the behavioral analysis, our results showed main effects of TMT and age that were characterized by fewer processed items during TMT-B in comparison to TMT-A and fewer processed items in participants over 66 years of age as compared to the younger group. Moreover, an interaction effect of age and TMT revealed significant differences between the age groups for the first (TMT-A/B versus TMT-C) and second (TMT-A versus TMT-B) Helmert contrast. Concerning the error rates, a reduced number of errors was observed for the TMT-A in older participants.

Regarding cortical blood oxygenation levels, a main effect for TMT was detected showing higher O_2Hb levels during TMT-A/B in comparison to TMT-C. Moreover, a main effect for age was observed in various ROIs, revealing higher O_2Hb levels for the older group.

When data were corrected for the number of processed items (O_2Hb/item), the

main effect for TMT was more pronounced than in the time-corrected analysis and manifested itself in four ROIs (left IFG, right IFG, left DLPFC, right DLPFC). While the time-corrected measurement only showed TMT effects for TMT-C contrasted to TMT-A/B, the item-correction additionally revealed significant second Helmert contrasts for TMT-A versus TMT-B. Contrary to the time-correction, the item-corrected data showed no age-related effect on brain activity during TMT performance.

Our results confirm previous research findings showing fewer completed items during the TMT in older volunteers (Hamdan & Hamdan, 2009; Muller et al., 2014; Rasmusson et al., 1998; Rodewald et al., 2012; Tombaugh, 2004). Several studies have shown that the execution time of the TMT increases with age, especially for TMT-B (Hamdan & Hamdan, 2009; Rasmusson et al., 1998; Rodewald et al., 2012). This fact was interpreted as age-related deterioration of mental flexibility, processing speed and attention (Drane et al., 2002; Tombaugh, 2004). Moreover, our results support previous findings of more completed items during TMT-A compared to TMT-B regardless of age (Gaudino et al., 1995; Muller et al., 2014). This is not surprising since TMT-B is considered the more difficult task requiring further cognitive resources as inhibition and set-shifting. For this reason, an increased age effect in TMT-B seems obvious.

Note that no higher error rate was observed in older age. Instead, we observed a reduced error rate in the older participants during TMT-A. Irrespective of the age group, the number of errors during TMT-A and TMT-C was lower than during TMT-B, confirming a higher degree of difficulty of the TMT-B. It could be argued that the reduction of the processing speed in old age explains a lower error rate in TMT-A, since cognitive deficits are compensated by slowing down according to a shift of the speed-accuracy trade-off (Zimmerman, 2011). However, in our data this hypothesis was not verified by a correlation between processing speed and error rates within older adults, a fact that could be due to the generally very low error rate. A study by Muller et al. (2014) could not determine any age effects with regard to the number of errors.

The current state of research assumes that TMT performance decreases with age, due to reduced processing speed and accuracy (Bäckman et al., 2004;

Hamdan & Hamdan, 2009; Mitrushina et al., 2005; Rasmusson et al., 1998; Tombaugh, 2004). A frequently discussed hypothesis is the compensation of age-related deficits by increasing activity and plastic reorganization of brain regions. According to previous research, an increasing bilateral activation (Muller et al., 2014) could be determined in high-performing older participants (Cabeza et al., 2002). It was shown that overactive regions in old age are responsible for performance success (Rossi et al., 2004), indicating increased cortical resources needed to solve a task. A study by Hagen et al. (2014) was able to determine a higher activity combined with a reduced performance in the right Broca's area and primary somatosensory cortex as well as in the left primary motor cortex in older participants. In line with our results, other studies report a frontal over-recruitment in old age (C. L. Grady, 2008; Spreng et al., 2010) to maintain executive functions.

As expected, our results showed a major effect for TMT in both the time-corrected and item-corrected version. Remarkably, however, the correction for processed items seems to highlight the effects between TMT conditions. Only the item-correction revealed differences between the hemodynamic response of TMT-A and TMT-B. Regarding this point, the results of previous studies are ambivalent. Whereas some studies found significant effects for TMT with a higher activation for TMT-B than for TMT-A (Shibuya-Tayoshi et al., 2007; Takeda et al., 2011), in some time-corrected test approaches, this TMT-effect could not always or only weakly be determined so far (Hagen et al., 2014; Muller et al., 2014). This observation was the basis for our previous study on the effects of speed and task complexity (Rosenbaum, Blum, et al., 2018). We assumed, since TMTA and TMTB differ in two dimensions – the complexity of the items and processed items, both aspects could have opposing effects on the hemodynamic response. In the original neurophysiological TMT experimental setup, the completion time is used as a primary performance metric. The goal is to complete both TMT subtasks as fast as possible and the time to completion is measured and recorded as a performance variable. Thus, if a block design is used, the performance variable time is omitted by limiting the working time to e.g., 30 seconds. To better elicit the effects between TMT-A and TMT-B, it is necessary to control for the processing

speed factor, since first, it is assumed that the blood oxygenation within a participant and TMT block is positively associated with the number of completed items. A second assumption is the positive association between blood oxygenation and task complexity. Normally in TMT-A more items are completed than in TMT-B (higher processing speed – higher O_2Hb levels) (Gaudino et al., 1995; Muller et al., 2014), whereas TMT-B, being the more difficult task (task complexity), also causes additional cortical activation. Consequently, both effects could obscure each other. This effect was confirmed in our previous study, where the results showed that the processing speed effects far outweighed the differences in task demands between TMTA and TMTB. From these two assumptions, we developed the correction method in our first study, which calculates the ratio of individual O_2Hb levels per TMT block and averaged performance (Rosenbaum, Blum, et al., 2018).

Not surprisingly, the effect for the TMT-A versus TMT-B contrast found in the item-controlled analysis, was characterized by a higher hemodynamic response during TMT-B in comparison to TMT-A. The different performance requirements of the sub-tests TMT-A and TMT-B are thus shown on a neurophysiological level. Our findings are in line with the findings of several studies that agree on large-scale bilateral frontal activation, with higher values in the TMT-B than in TMT-A (Kubo et al., 2008; Shibuya-Tayoshi et al., 2007; Takeda et al., 2011). Other studies specified the region affected by a higher activity during TMT-B compared to TMT-A as the area of DLPFC (Hagen et al., 2014; Jacobson et al., 2011; Muller et al., 2014). In comparison to TMT-A, dual-task paradigms such as the TMT-B, where different tasks (number series and alphabet) have to be executed simultaneously, especially require executive functions as cognitive flexibility.

Contrary to our expectations, the item-corrected analysis showed no effect of age. This result was surprising as the older participants completed fewer items than the younger participants did and therefore the ratio calculation was suspected to reveal a higher activity for the older group. Interestingly, the blood oxygenation per item did not differ between the age groups, while the total blood oxygenation in the time-corrected data showed higher values within the older group in the ROIs of left DLPFC and right SAC. Consequently, higher O_2Hb levels

within the older participants in the time-corrected version could be interpreted as a compensation mechanism on the one hand, but on the other hand the validity of the statement must also be questioned, since time-correction in contrast to the item-correction does not take the individual performance into account. A more detailed investigation showed a linear negative correlation between age and number of completed items and a negative quadratic correlation between age and O_2Hb levels in lDLPFC and rSAC. The quadratic relationship revealed a turning point at the age of 65-70 years, which was characterized by an increase of O_2Hb levels up to 65-70 years and a subsequent decrease. Apart from the frequently used interpretation of increasing cortical blood oxygenation as age-related compensatory effect (Cabeza et al., 2002; Muller et al., 2014; P. A. Reuter-Lorenz et al., 2000; Rossi et al., 2004), various other neuroscientific models, as the idea of age-related increase in O_2Hb levels due to lower efficiency and specificity (Alexa M. Morcom & Henson, 2018), the dedifferentiation hypothesis (Lindenberger & Baltes, 1997) and the CRUNCH-model (Patricia A Reuter-Lorenz & Cappell, 2008) contribute to the understanding of cognitive aging. It is worth highlighting the CRUNCH model, that assumes an additional recruitment of cortical resources with increasing task complexity until the age-dependent personal load limit is reached. However, the capacity of additional resources is limited and subsequently performance and brain activation drop again. What our data reveal is a decline in cortical activity starting at about age 65-70 years. It is thinkable that this age group already reaches their individual functional processing limit when performing TMT. Furthermore, the concept of neural dedifferentiation in the ageing brain also explains the results found, as age-related over-recruitment in the context of reduced performance has often been interpreted as a sign of neural dedifferentiation (de Chastelaine et al., 2011; A. M. Morcom et al., 2007). The assumption is based on the idea that older subjects, due to a lower specificity of brain regions, activate several functional areas together, which in younger subjects are specialized to only one cognitive functionality.

Interestingly, the analysis to control for medication (antiplatelets/anticoagulants/blood pressure medication) showed an effect in the

item-corrected data, whereas the time-corrected results remained unaffected. Therefore, a direct influence of medication consumption on the fNIRS signal is unlikely. Nevertheless, note that only by adding the medication as a covariate, an age effect became apparent in the item-corrected data. The effect may be due to a positive influence of antihypertensives (Levi Marpillat et al., 2013) and anticoagulants (Friberg & Rosenqvist, 2018) on cognitive function, explaining a highlighting of age differences, as the older participants showed higher medication consumption compared to the younger group.

Finally, some limitations have to be considered.

First, our results raise the question of the limitation of our correction method. The decisive point is that the correction method introduced corrects for performance and since TMT performance is associated with age, it would also partially correct for age effects or, more precisely, for the part of the age-related variance in brain activity that is related to performance. With respect to the used correction method, one might argue that the correction of the O_2Hb levels for the number of processed items consequently leads to higher blood oxygenation levels when participants show a reduced performance. However, this conclusion assumes that blood oxygenation would stay stable if volunteers work with less effort, which has been shown to not be the case (Rosenbaum, Blum, et al., 2018). On the one hand, the integration of processed items acts as efficiency measure, which is not considered in most other studies (Hagen et al., 2014; Muller et al., 2014). On the other hand, it could be argued that the ratio approach is too simplified to provide a well-founded result. However, we were able to demonstrate in our previous study (Rosenbaum, Blum, et al., 2018) that a correction of the fNIRS data by a regression approach (in which the average number of items was regressed out of the activity level for each individual at three different speed levels) and the simple ratio approach produced similar results. Since not every study can collect TMT data at three different speed levels, it provides a simple approximation. It should also be emphasized that our correction method is not intended to replace the original TMT measurement, which is not time-limited, nor to replace the time-corrected analysis for block designs. In case block designs are used, it is only

intended to highlight performance differences as an additional index, which, as you can see from our results, should not be underestimated.

A second point to consider is the interpretation of the increase in hemodynamic response as a compensatory pattern. Although there is evidence in the literature for a compensatory nature of the increase in O_2Hb levels in older subjects (Cabeza et al., 2002; Du et al., 2016; C. L. Grady et al., 2005), research varies greatly and the concept of age-related compensation was also critically questioned (Colcombe et al., 2005; Duverne et al., 2009; Höller-Wallscheid et al., 2017). For example, Höller-Wallscheid et al. (2017) found an age-independent additional recruitment of cognitive resources from the contralateral hemisphere when participants where challenged by higher task demands, reflecting an adaptation to the increased requirements rather than a compensation for age deficits. Note that our results are only intended to represent one possible proposed interpretation. Other models of cognitive aging are also conceivable and have been discussed comparatively.

Third, despite many advantages (e. g. seated working, tests in realistic environment, social interaction...), the fNIRS emitters have a limited spatial resolution and a low penetration depth into the cranial calotte of 2-3 cm (Haeussinger et al., 2011). The near-infrared radiation therefore only detects superficial cortical structures and exhibits inter-individual variability depending on the path length and tissue composition of the grey matter.

The fourth limitation concerns the age range, since our study compared middle-aged and older participants. A comparison with a younger cohort (<50 years) would probably have highlighted the results. However, it was precisely our concern to detect subtle differences in the incipient neurodegenerative processes, which we suspected to originate in the age of early retirement. One could criticize that the evaluation method we have chosen with separation of the cohort into two groups via a median split only represents a simplification. For this reason, we additionally checked the age effects in a continuous evaluation within the exploratory analysis.

Another point that needs explanation is the high error variance visible on figures 9 and 10. This can be explained by an increased heterogeneity in performance,

which is particularly evident in a high-aged group of participants. Furthermore, due to the tight schedule of the TREND study, the study at hand only includes a restricted TMT block number of two blocks per subject, which further increases the variability.

Finally, data were collected in a naturalistic observational study without examination of neurodegenerative marker such as amyloid-β or Tau in CSF or amyloid-β- or Tau-PET or MRI. For that reason, no assigning to a sub-group of participants with pre-clinical neurodegeneration was possible.

2.2.5. Conclusion

To the authors' knowledge, this is the first study to examine the influence of age on TMT performance using a comparison of a time-correction and an item-correction method. To sum it up, our findings confirm several important age-related effects: fewer completed items, a lower error-rate during TMT-A and increased cortical activity in older participants. Our results emphasize the importance of applying a correction methods for the performance variable, since significant performance deficits exist within the older group. By slowing down the processing speed, deficits could be compensated according to a shift of the speed-accuracy trade-off. Contrary to our forecasts, the item-correction did not increase the sensitivity to age effects. Of particular importance, however, is the fact that the contrast between the TMT conditions TMT-A and TMT-B only became apparent after application of the correction method.

The ratio calculation can only be evaluated as a simplified approach to account for the individual performance and future research could aim to improve the correction method. Nonetheless, our comparison clearly shows that the number of processed items in TMT has a decisive influence on the overall capacity and should not be underestimated in the commonly used time-corrected designs.

2.3. Study 3 - Effects of ageing on functional connectivity in a neurodegenerative risk cohort: Resting state versus task measurement using functional near-infrared spectroscopy

The study was approved by the Ethics Committee of the University of Tuebingen (90/2009BO2).

The contents of this chapter are published:

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Abstract:

Changes in functional brain organization are considered being particularly sensitive to age-related effects and may precede structural cognitive decline. Recent research focuses on ageing processes determined by resting state (RS) functional connectivity (FC), little is known about differences in FC during rest and cognitive task conditions in elderly participants.

The purpose of this study is to compare FC within and between the cognitive control (CCN) and dorsal attention network (DAN) at RS and during a cognitive task using functional near-infrared spectroscopy (fNIRS). In a matched, neurodegenerative high-risk cohort, comprising early (n=98; 50-65y) and late (n=98; 65-85y) elder, FC was measured at RS and during performance of the Trail Making Test (TMT) via fNIRS.

Both, under RS and task conditions our results revealed a main effect for age, characterized by reduced FC for late elder subjects within the left inferior frontal gyrus. During performance of the TMT, negative correlations of age and FC were confirmed in various regions of the CCN and DAN. For the whole sample, FC of within-region connections was elevated, while FC between regions was decreased at rest. The results confirm a reorganization of functional brain connectivity with increasing age and cognitive demands.

2.3.1. Introduction

It is well known that ageing is associated with neurodegeneration. However, natural ageing processes are still far from being comprehensively understood. A major challenge lies in the distinction between physiological and pathological ageing, since the brain changes its structure and thus also its function during the entire lifetime (Fjell et al., 2014; Hagen et al., 2014). At the cognitive level, ageing is associated with a decrease in executive functions, e.g. processing speed, working memory and mental flexibility (Hedden & Yoon, 2006). On a neuropathological level, tissue reduction of grey and white matter (Bergfield et al., 2010; Buckner, 2004; Courchesne et al., 2000; Giorgio et al., 2010; Good et al., 2001; Madden et al., 2009; Pfefferbaum et al., 1994; Walhovd et al., 2005), loss of synaptic connections (Terry & Katzman, 2001) and amyloid deposition in non-demented individuals (Pike et al., 2007) have been observed in old age. Accordingly, an essential focus of research is the identification of correlations between cognitive and structural neuronal changes. In addition to anatomical remodeling, a shift of functional connections in old age has also been described, e.g. various patterns of hypo- or hyper-recruitment of brain regions have been observed during the senium (Cabeza, 2002; Logan et al., 2002; Park & Reuter-Lorenz, 2009; Reuter-Lorenz, 2002). It was even hypothesized that functional changes precede the structural reorganization of the brain (Jack et al., 2010). Changes in functional brain organization are therefore considered to be particularly sensitive to early age-related effects. First described by Friston et al. (1993), functional connectivity (FC) is defined as the temporal relationship between spatially separated neurophysiological processes. These functional connections between different brain areas exist both at rest and during task accomplishment and are considered as functional networks such as the cognitive control network (CCN), dorsal attention network (DAN), default mode network (DMN) and salience network (SN) (Grefkes et al., 2013). In the past, it has already been shown that FC differs between subjects with mild cognitive impairment (MCI) or Alzheimer's disease (AD) and healthy controls (Binnewijzend et al., 2012; Dai et al., 2014; Sorg et al., 2007; Wang et al., 2007). Within the clinical context, a malfunctioning of especially the CCN and DAN has been connected to

psychiatric disorders such as depression (Respino et al., 2020; Rosenbaum, Hilsendegen, et al., 2018) as well as Alzheimer's Dementia (Li et al., 2018). In addition, there is evidence for changes in the FC pattern with increasing age. The consensus of ageing studies indicates a decrease in FC within resting state (RS) networks such as the default mode network (DMN) or the salience network (SN) (Chan et al., 2014; Ferreira et al., 2016; Geerligs et al., 2015; Grady et al., 2016) concomitant with an increase in FC between the different networks (Chan et al., 2014; Ferreira & Busatto, 2013; Ng et al., 2016) as well as a general association of ageing with reduced global efficiency and modularity (Varangis et al., 2021). Moreover, Esposito et al. (2018) described a reduction of the physiological anticorrelation activity between the DMN and the DAN in RS as part of a normal aging process and MCI as a status in which these changes are even more pronounced. Similar observations have on principle also been made during different tasks (Spreng et al., 2016). However, in most studies on ageing processes, FC has been determined by RS measurements (Betzel et al., 2014; Chan et al., 2014; Ferreira & Busatto, 2013; Schlee et al., 2012; Siman-Tov et al., 2017; Zonneveld et al., 2019) as this is easier to implement in clinical experimental setups. Indeed, we hypothesized that deficits associated with higher age as well as early stages of neurodegeneration, for which age actually is the main risk factor, would initially show up as reduced performance and/or altered neural functionality in the management of cognitive tasks. Therefore, we compared the measurement of FC via functional near-infrared spectroscopy (fNIRS) during resting state (rsFC) with a measurement during execution of the Trail Making Test (TMTFC), a neuropsychological task for the assessment of executive (i.e., frontal lobe) functions (Arbuthnott & Frank, 2000).

In particular the TMT-B subtest requires activation of frontal cortical structures such as the inferior and middle frontal cortex as well as the dorsolateral prefrontal cortex (DLPFC) (Hagen et al., 2014; Jacobson et al., 2011). Performing the TMT therefore induces an activation of the CCN as well as the DAN, as it activates the dorsal parts of the lateral prefrontal cortex (DLPFC), cingulate cortex (dACC = dorsal anterior cingulate cortex) and parietal cortex/somatosensory association cortex (Breukelaar et al., 2017; Rosenbaum, Maier, et al., 2018; Vossel et al.,

2014). We chose fNIRS as it not only exhibits the particular advantage of being applicable to participants sitting at a desk in an upright position to perform the task (including hand/arm movements) under natural conditions (Ehlis et al., 2014), but also because it is known to reliably reflect cortical activity in the above named areas in an elderly cohort (Hofmann et al., 2021; Rosenbaum, Hilsendegen, et al., 2018).

The study at hand thus aims to improve our understanding of FC during task completion in contrast to RS measurements for the purpose of early detection of age-related changes indicative of incipient neurodegenerative processes. To this end, we investigated two groups of elderly participants, early (50 to 65 years of age) versus late elders (65 to 85 years of age) enriched with but also matched for other neurodegenerative risk factors like REM sleep behavior disorder (RBD) or depression. The rationale behind the chosen cut-off at 65 years was the following. If an individual develops a neurodegenerative dementia earlier than 65 years of age, the particular diagnosis (e.g. AD) is indicated as *early-onset* and the etiological background (e.g. genetic factors) is rather complex in many cases (Kuruppu & Matthews, 2013). After the age of 65 years, the development of a neurodegenerative disorder like AD becomes more and more frequent and is therefore in most cases considered as senile. Even within the current version of the International Classification of Diseases by the World Health Organization (WHO) (2018), this diagnostic cut-off at the age of 65 years is used. Our first goal was to determine the performance of the late elder participants in comparison to the early elder group based on the number of items completed during TMT execution. Secondly, potential differences should be investigated between rsFC and TMTFC, again within the late compared to the early elder subgroup. In this regard, we were especially interested in the FC patterns during TMT-B execution in contrast to RS since this subtask is considered most demanding and therefore might serve as a sensitive marker for subtle cognitive decline. In general, we assumed the early elder subjects to perform worse in the TMT compared to the early elders. Irrespective of age, we presumed that FC would be higher during task completion than at rest.

2.3.2. Participants and Methods

Study population

The participants originated from the *Tuebinger evaluation of risk factors for early detection of neurodegeneration* (TREND)-study database. This is a large-scale study from the Department of Neurology and the Department of Psychiatry and Psychotherapy of the University Hospital of Tuebingen, Germany, initiated in 2009, which aims at investigating possible prodromal markers for neurodegenerative diseases (Berg, 2012; Heinzel et al., 2013; Hobert et al., 2011). The study was approved by the Ethics Committee of the University of Tuebingen and is in accordance with the standards of the World Medical Associations Declaration of Helsinki. Informed consent was obtained from all participants included in this study. The TREND study is conducted via biennial assessments. Inclusion and exclusion criteria initially were as follows: age between 50 and 80 years, no neurodegenerative disease at baseline and – if applicable – at least one of the following prodromal markers for neurodegeneration: depression, hyposmia and RBD, characterized by loss of physiological atonia during REM sleep. Especially individuals suffering from the latter have a risk of about 50% to develop Parkinson's Disease or dementia within ten years (Postuma & Montplaisir, 2009). Individuals who did not experience any of these symptoms were recruited as controls which concerns nearly half of the participants. The assessment battery includes medical history, neurological examination, transcranial sonography, olfactory, autonomic and cognitive testing with the CERADplus battery (Consortium to Establish a Registry for Alzheimer's Disease) (Morris et al., 1988) and MOCA (Montreal Cognitive Assessment) (Nasreddine et al., 2005) as well as self-report questionnaires assessing RBD, mood (Beck depression inventory version I) as well as quality of life (for more details visit: https://www.trend-studie.de/). Study data are collected and managed using REDCap electronic data capture tools hosted at the University of Tuebingen (Harris et al., 2009).

In the study at hand, a subsample from the TREND cohort of in total n = 196 participants (50–85 years of age) has been investigated, comprising n = 98 late

(> 65 years of age) as well as n = 98 early elders (< 65 years of age), matched according to education, gender, and risk-factors for neurodegenerative diseases and/or cognitive decline (amnestic MCI (aMCI) and RBD). The early elder group consisted of 54% female participants, had a mean age of 60.23 years (*SD* = 2.98) and a mean education of 14.23 years (*SD* = 2.44). Within the late elder cohort, 43% of participants were female, the mean age was 70.27 years (*SD* = 4.46) with on average 13.96 years (*SD* = 2.69) of education. Due to the matching procedure, the early and late elder subgroup did not differ regarding the frequency of neurodegenerative risk factors (aMCI: $\chi^2(1) = 1.71$, $p = 0.19$, RBD: $\chi^2(1) = .02$, $p = 0.89$), years of education ($t(194) = 0.75$, $p = .453$, $d = .11$) or gender ($\chi^2(1) = 2.47$, $p = 0.116$). 87% of the participants were on medication, in particular blood pressure medication (41%), anticoagulants (20%) and antidepressants (11%). The early and late elder sample did not differ in terms of their medication status ($\chi^2(1, n = 196) = 3.71$, $p = .054$) (table 5).

Table 5: Epidemiological data of the investigated cohort.

aMCI = amnestic mild cognitive impairment, RBD = rapid eye movement sleep behavior disorder, SD = standard deviation.

*Mini Mental State Examination (MMSE)

**depressive characteristics according to Beck's Depression Inventory (BDI-I)

***Consortium to Establish a Registry for Alzheimer's Disease (CERAD) total score (Chandler et al., 2005)

*****Logical Memory (LM) subtest of the Wechsler Memory Scale-IV (WMS-IV), I = direct recall, II = delayed recall)*

Characteristics	**Early elders**	**Late elders**	Statistics
Age (mean years)	60.23 (SD = 2.98)	70.27 (SD = 4.46)	-
Gender (% female)	54.10	42.90	p = 0.11
Education (mean years)	14.23 (SD = 2.44)	13.96 (SD = 2.69)	p = 0.45
aMCI (% diagnosed)	9.20	15.30	p = 0.19
RBD (% diagnosed)	46.90	48.00	p = 0.89
Medication (% intake)	82.70	91.80	p = 0.05
MMSE* (mean score, SD)	28.47 (SD = 1.46)	28.25 (SD = 1.57)	p = 0.31
BDI (mean score, SD)**	7.84 (SD = 9.39)	8.02 (SD = 8.80)	p = 0.88
Global cognition* (CERAD mean total score, SD)**	88.24 (SD = 5.94)	81.79 (SD = 8.73)	p < 0.001
Episodic memory** (WMS-IV, I/II percentile rank)**	55.68 / 59.89	42.32 / 57.99	p = 0.003 / p = 0.67

Trail Making Test

The TMT as subtask of the CERAD-Plus test battery (Morris et al., 1988) is a standardized neuropsychological test procedure for the detection of cognitive deficits, especially checking for executive functions, working memory and mental flexibility (Arbuthnott & Frank, 2000). The modified version of the TMT used in our study consists of three sub-tests: TMT-A, TMT-B and a so-called TMT-C. Each of the sheets contains 25 items. During the TMT-A, subjects are instructed

to link randomly distributed numbers in an ascending order as quickly as possible. In the TMT-B, numbers and letters must be connected alternately according to the ascending number chain and alphabet. In addition to motor speed, visual search function and working memory, the TMT-B also tests the ability of task-switching. The TMT-C was developed as a control condition for functional imaging to simply assess motor activity by tracing pre-drawn lines. In order to minimize disturbing environmental influences, the measurement took place in a quiet, darkened room. The participants were instructed to sit upright and avoid head movements during simultaneous fNIRS measurement. Physiological working posture was ensured by an inclined desk. The TMT was performed in a block design as follows: TMT-A–TMT-B–TMT-C–TMT-A–TMT-B–TMT-C–TMT-A–TMT-B. Blocks were separated by a 30-seconds pause. Implementation started with a five-minutes resting measurement with closed eyes, then the first two blocks were conducted. After a short instruction and exercise, the test was performed without a time limit to ensure a later standardization of analysis for the TMT behavioral data. During the subsequent six blocks, the completion time was limited to 30 seconds. The number of items and errors was documented for each participant.

fNIRS and preprocessing of data

The concentration of oxygenated hemoglobin (O_2Hb) during brain activation was measured by a continuous wave, multichannel fNIRS system with a sampling rate of 10 Hz. A total of 38 channels were measured (24 fronto-temporal, 14 parietal; figure 11) with fixed inter-optode distances of 30 mm (no short-distance channels were included). Exact anatomical fixation was performed using an optode holder cap with reference points F3/F4 and Fp1/Fp2 (fronto-temporal) and C3/C4 (parietal) (Homan et al., 1987). Corresponding brain areas of each channel were extrapolated from reference points as in the work by Singh et al. (2005) as well as by other colleagues (Tsuzuki & Dan, 2014; Tsuzuki et al., 2007) based on the Colin 27 template. Data were recorded with a semiconductor laser and avalanche diodes at two wavelengths (695 ±20 and 830 ±20 nm) with 4.0±0.2 mW for each wavelength at each optode. The acquisition and pre-processing of the

measurement data was performed with the software of the ETG-4000 (ETG-4000 Optical Topography System; Hitachi Medical Co., Japan).

Data were assessed during the 5-minutes RS measurement and under subsequent TMT performance. We ensured proper time-locking between fNIRS acquisition and the TMT task by the investigator pressing a trigger button and simultaneously requesting the subject to start the task. The 30-seconds blocks for each condition were averaged with a 10-seconds baseline correction and a linear detrending. In more detail, we decided to average the different trials and then compute the FC of the hemodynamic courses, as this approach reduces the background noise.

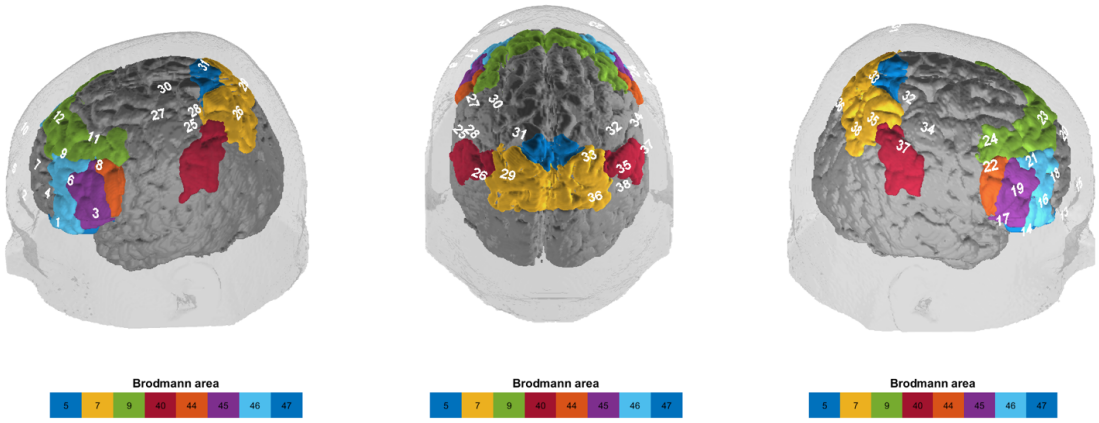

Figure 11: fNIRS: the white numbers above on the cortical surface represent the channel denotation, whereas the black numbers below within the colored boxes correspond to the Brodmann areas (left - back - right view).

Data-Analysis

For data analysis MATLAB R2016b (MathWorks Inc., Natick, USA) was used. We corrected for high amplitude movement artifacts through TDDR correction (Fishburn et al., 2019) and the method of bandpass filtering (0.01–0.1 Hz) to filter out very high or low frequency artifacts and a correlation-based signal improvement to reduce motion influences on O_2Hb-levels (Cui et al., 2010). In further processing an independent component analysis (ICA)-based reduction of masticatory artifacts was performed. Artifact-loaded channels that outlasted

preprocessing were visually detected and interpolated by surrounding channels. If more than 10% of the channels showed artifacts, the subject was excluded (in total n=8 subjects). Finally, a global signal correction was performed by means of a PCA based gaussian kernel filter (Zhang et al., 2016). The preprocessing steps were based on the guidelines of Brigadoi et al. (2014).

For the computation of connectivity during task performance, data were averaged for each condition of the TMT. Furthermore, a baseline correction was performed where the activation during baseline measurement was subtracted from the activation during task completion. FC was calculated as Pearson correlation coefficients after the data of each channel pair was checked for multivariate outliers by malahanobis distances (Arbuthnott & Frank, 2000). After FC indices were computed for each channel pair, FC between regions and within regions was computed by averaging the FC indices of the corresponding channels (e.g. all channels FCs of the left DLPFC to the right DLPFC).

Statistics

After preprocessing, we compared the FCs within and between pre-defined region-specific nodes within the CCN and DAN: the somatosensory association cortex (SAC), dorsolateral prefrontal cortex (DLPFC) and inferior frontal gyrus (IFG). We computed FC for “within” and “between” region connections, either short-distance (ipsilateral) or long-distance, i.e. connections to contralateral regions (Zhu et al., 2017). Statistical data evaluation was performed with IBM SPSS Statistics Version 24.

We calculated repeated measures MANOVAs with the factors

a) resting state and TMT (levels: RS, TMT-C, TMT-A, TMT-B; within-subjects),
b) region of interest (ROI): 21 levels, each left (l), right (r):

either between: lDLPFC_lIFG, lDLPFC_rIFG, lDLPFC_rDLPFC, lDLPFC_rSAC, lDLPFC_lSAC, rDLPFC_lIFG, rDLPFC_rIFG, rDLPFC_rSAC, rDLPFC_lSAC, lIFG_rIFG, lIFG_rSAC, lIFG_lSAC, rIFG_rSAC, rIFG_lSAC, rSAC_lSAC

or within: rSAC_within, lSAC_within, lDLPFC_within, rDLPFC_within, lIFG_within, rIFG_within

and

c) age (<65 and >65 years; between-subjects).

Moreover, we investigated each ROI separately by a repeated ANOVA (levels: RS, TMT-C, TMT-A, TMT-B; within-subjects) and age (< 65 and > 65 years; between-subjects).

Post-hoc analysis included simple contrasts (RS versus TMT-C, RS versus TMT-A, RS versus TMT-B) corrected by the Benjamini-Hochberg procedure, and Helmert contrasts (average TMT-A and TMT-B [TMT-A/B] vs TMT-C; TMT-A vs TMT-B).

2.3.3. Results

Behavioral results

General performance and age. Post hoc analysis of Helmert contrasts confirmed fewer processed items during TMT-A/B in comparison to TMT-C ($F(1, 194) = 1227.80$, $p < .001$, $\eta^2 = 0.86$), fewer processed items during TMT-B in comparison to TMT-A ($F(1, 194) = 1244.60$, $p < .001$, $\eta^2 = 0.87$) and fewer processed items in the late compared to the early elder subjects (table 6).

Interaction between age and task level. A two (age: early versus late elder subjects) by three (TMT: TMT-C versus TMT-A versus TMT-B) ANOVA showed a significant main effect of TMT ($F(2, 388) = 1236.95$, $p < .001$, $\eta^2 = 0.86$) and a main effect of age ($F(1, 194) = 25.81$, $p < .001$, $\eta^2 = 0.12$). Moreover, the interaction of age and TMT ($F(2, 388) = 13.83$, $p < .001$, $\eta^2 = .07$) reflected that the age groups showed significant differences during TMT-A/B in comparison to TMT-C ($F(1, 194) = 28.72$, $p < .001$, $\eta^2 = 0.13$) but not between TMT-A and TMT-B ($F(1, 194) = 1.38$, $p = .241$, $\eta^2 = .01$).

The sexes did not differ in terms of the number of processed items (TMT-A: $t(194) = - 1.25$, $p = 0.212$, $d = 0.20$), (TMT-B: $t(194) = 0.16$, $p = 0.875$, d = .02).

Table 6: Demographics and behavioral data: Number of processed items during TMT-A, TMT-B and TMT-C, depending on age. TMT = trail making test, SD = standard deviation.

		Early elder subjects (< 65 years)		Late elder subjects (> 65 years)		
		mean	*SD*	mean	*SD*	*Statistics*
Age		60.23	2.98	70.27	4.46	-
TMT-A	processed items	22.31	3.16	19.49	4.68	$p < 0.001$
TMT-B	processed items	11.62	4.02	9.50	3.49	$p < 0.001$
TMT-C	processed items	23.85	0.94	23.87	0.60	$p = 0.86$
TMT-B/A	processed items	0.53	0.18	0.51	0.26	$p = 0.62$

fNIRS

Interaction between task level and ROI. A repeated measures MANOVA revealed differences between the four measurement conditions (RS, TMT-C, TMT-A and TMT-B) concerning the ROI ($F(63.00, 1678.40) = 8.24$, Wilk's $\Lambda = .45$, $p < .001$, partial $\eta^2 = .24$). Concerning the FC within and between individual ROIs of the CCN and DAN, we observed multiple effects for the TMT. Between-regions differences were characterized by lower FC in resting state than during TMT-A, -B and -C. In contrast, within-region effects were characterized by significantly higher FC at rest than during the TMT-A, -B and -C (table 7, figure 12). For corresponding correlation matrices, please see our supplemental figure 21.

Table 7: Between- and within-region FC in RS versus TMT task conditions. FC = functional connectivity, DLPFC = dorso-lateral prefrontal cortex, IFG = inferior frontal gyrus, SAC = sensory association cortex, l = left, r = right.

Between-region FC		Statistics
	lDLPFC_lIFG	F (2.74, 531.03) = 10.40 p < .001, η^2 = .05
	lDLPFC_rIFG	F (3, 582) = 11.06 p < .001, η^2 = .05
	rDLPFC_lIFG	F (2.87, 556.89) = 9.21 p < .001, η^2 = .05
	rDLPFC_rIFG	F (2.89, 560.38) = 13.56 p < .001, η^2 = .07
	lIFG_lSAC	F (3, 582) = 4.48 p = .042, η^2 = .02
	lIFG_rSAC	F (3, 582) = 4.31 p = .035, η^2 = .02
	rIFG_lSAC	F (3, 582) = 7.86 p < .001, η^2 = .04
	rIFG_rSAC	F (3, 582) = 9.26 p < .001, η^2 = .05
Within-region FC		
	lDLPFC	F (3, 582) = 13.08 p < .001, η^2 = .06
	lSAC	F (3, 582) = 7.23 p < .001, η^2 = .04

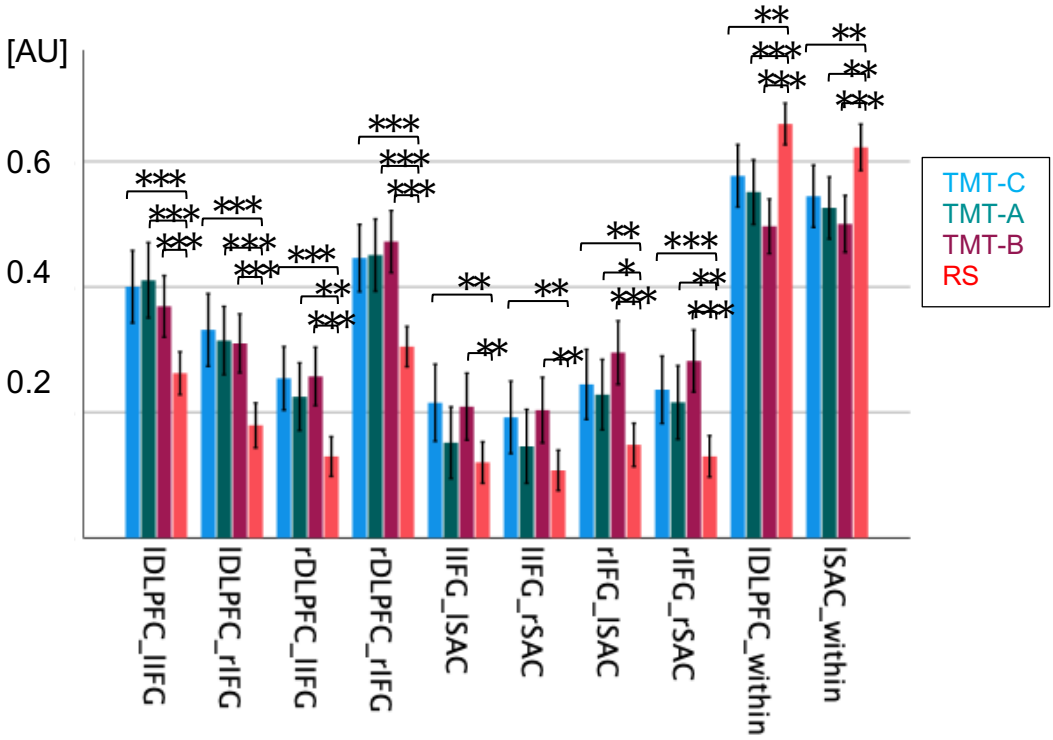

Figure 12: Mean between- (first eight bar groupings) and within-region (last two bar groupings) FC during RS versus the different TMT task conditions. Error bars indicating the 95% confidence interval. Asterisk representing the statistically significant difference between the RS and different task conditions for all the regions shown (* for p <.05, ** for p <.01 and *** for p >.001), see table 7 as well as supplemental table 9. *RS = resting state, AU = arbitrary unit, TMT = trail making test, DLPFC = dorso-lateral prefrontal cortex, IFG = inferior frontal gyrus, SAC = sensory association cortex.*

Interaction between age, task level and ROI. Between-subjects, a main effect for age indicated lower FC within the ROI of the left IFG for the late as compared to the early elder subjects (lIFG_within: F (1, 194) = 10.17, p = .042, η^2 = .05), independent of the corresponding condition (RS versus TMT). Moreover, we observed significant negative correlations of FC and age in various ROIs under the different TMT task conditions: During the TMT-A in the lIFG_within (r = - 0.22, p = .002) as well as between lIFG_rIFG (r = - 0.15, p = .040), during the TMT-B in the lIFG_within (r = - 0.16, p = .025) (figure 13) and during the TMT-C in the lDLPFC_within (r = - 0.14, p = .046).

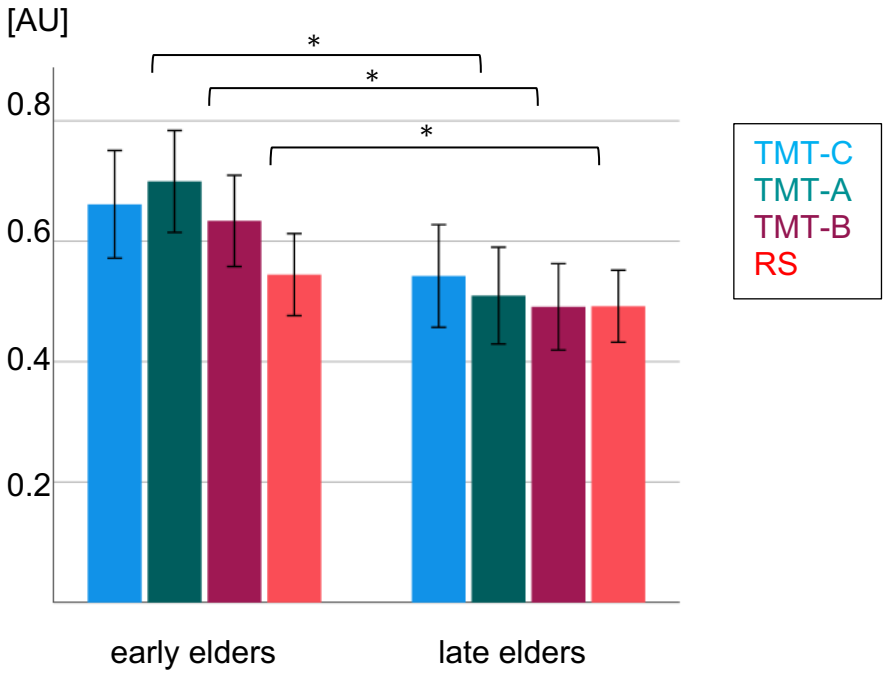

Figure 13: FC within the left IFG during RS versus the different TMT task conditions. early elders: participants < 65 years, late elders: participants > 65 years. Error bars indicating the 95% confidence interval. *Asterisks represent a statistically significant difference between the early and the late elder subgroup ($p < 0.05$).

RS = resting state, TMT = trail making test, AU = arbitrary unit.

2.3.4. Discussion

The study at hand aimed to explore the differences of FC during TMT task completion and RS measurements depending on age (early versus late elder subjects) within a cohort at high risk for neurodegeneration. In summary, our results emphasize age-related task performance decline to be associated with changes of brain network organization, as it has on principle been described before (Varangis et al., 2019). Regarding the behavioral results, we generally confirmed fewer processed items during the TMT-A/-B in comparison to the TMT-C and fewer processed items during the TMT-B in comparison to the TMT-A. As expected, we found a reduced working speed with fewer processed items for the late elder as compared to the early elder subjects, which is in line with numerous

previous studies (Hamdan & Hamdan, 2009; Rasmusson et al., 1998; Rodewald et al., 2012). In more detail, our results showed that this age-related difference of task performance was observed in comparison of the TMT-A/-B and TMT-C but not in comparison of the TMT-A and TMT-B. Therefore, it seems that the TMT-B, although being the cognitively more demanding task, might not be challenging enough to unmask age-related deficits earlier than the TMT-A. Alternatively, one could argue that the cognitive domain affected by age-related changes is indeed not primarily the executive function but rather general processing speed, though not simple motor velocity as both groups perform comparable at the TMT-C.

Most previous studies investigated age-related effects on FC during RS measurements (Betzel et al., 2014; Chan et al., 2014; Ferreira & Busatto, 2013; Schlee et al., 2012; Siman-Tov et al., 2017; Zonneveld et al., 2019). Herefrom, it is a known phenomenon that on principle older compared to younger individuals show a higher between- as well as lower within-network FC during RS (Chan et al., 2014; Grady et al., 2016; Spreng et al., 2016), whereas Chan et al. (2014) even correlated this observation with reduced episodic memory scores across the adult lifespan. This seems to contradict our findings as we described a higher within- and lower between-network FC during RS within an older population, though, importantly, in comparison to a task performance condition. Moreover, a four-year, long-term MRI study by Ng et al. (2016) more precisely described the increase of between-network FC changes with advancing age to be preceded by an initial decrease. Fittingly, the opposite effects for within-network FC, e.g. the DMN, have been described in the sense of an initial increase, followed by a later decrease (Bai et al., 2011; Damoiseaux et al., 2012). It remains questionable whether this is a compensation mechanism that attempts to maintain the status quo with an initial hyper-recruitment. Within this context, it further has to be stated that the individuals investigated within previous studies were even older compared to our study cohort as a whole (e.g. Grady et al. (2016): mean 69.0 years) which is why one could argue they were situated at a different point on this timeline.

An ageing-study on the comparison of FC during RS and TMT has not been reported yet, but there have been published approaches to compare FC between

RS and other forms of task conditions, mainly in the context of fMRI studies. For instance, Di et al. (2017) and Cole et al. (2014) reported within-network connectivity to be decreased as well as between-network connectivity to be increased during task performance compared with RS, which agrees with our results. An MRI study by Arbabshirani et al. (2013) compared the functional network connectivity during RS and performance of an auditory oddball task. Their results during task performance, however, showed a global decrease of FC concomitant with an increased activity in particular networks. They suggested that successful performance of a certain task may be facilitated by an increased recruitment of related brain regions rather than collaboration among different networks, which, again, somehow contradicts our findings, whereat it is important to remark that the work of Arbabshirani et al. (2013) was not an ageing study. With respect to FC, we identified discrepancies between brain regions depending on the mental state (RS versus TMT-C/-A/-B) as well. But interestingly, as described above, FC *within* regions of the CCN and DAN was higher at rest than during task performance, whereas FC *between* regions was higher during TMT performance compared to RS. Further results of a higher FC during RS in comparison to the task condition, similar to Arbabshirani et al. (2013), have already been reported by other authors (Fransson, 2006; Greicius et al., 2003; Hasson et al., 2009; Nir et al., 2006). Another possible explanation for this phenomenon might be the suppression of spontaneous thoughts by the attention demanding task (Fransson, 2006). Nevertheless, the current state of research is divided. Since other authors agreed on higher FC during task performance in comparison to RS measurements (Elton & Gao, 2015; Harrison et al., 2008; Shirer et al., 2012), an influence of the cognitive task domain on FC magnitude and organization seems obvious. Actually, this has been described repeatedly within the fMRI studies of Varangis et al. (2021, 2019), e.g. in the sense of a higher age-related effect on FC measures during fluid reasoning compared to an episodic memory task. However, all these observations can serve to better classify the partly contradictory research results. Further, from a methodical point of view, one has to consider that the systematical performance of RS before the TMT task measurements may represent a confounding factor. Albeit the inverted

order seems to be even more problematic, as in that case one would expect the probands to reflect during RS on their performance during the preceding task. Finally, an alternative view is provided by recent studies that suggest FC as a unique pattern that differs between individuals regardless of mental status including RS and task performance performance (Gratton et al., 2018; Noble et al., 2017).

When the variable age was taken into account within our investigation, a negative correlation between age and FC became visible in several ROIs; this on principle was the case during both, RS as well as the different forms of TMT performance, even though more pronounced under the task condition, as it has been hypothesized by us. In this regard, we mainly found within-networks effects, namely within the left hemisphere for the IFG and DLPFC. Furthermore, bilateral connectivity between the right and left IFG also was reduced. Hence, it seems that mainly reduced within-network FC, especially in the ROI of the left IFG, is associated with a reduced cognitive performance in advanced age. Interestingly, for the specific regions in which an age effect showed up within our investigation other authors even described a regional hypometabolism in FDG-PET in the context of different forms of early dementia and subsequent especially executive deficits (Schroeter et al., 2012). We consciously selected our aging cohort on the whole to exhibit even other high-risk factors for neurodegeneration (e.g. RBD and/or depression). Importantly, the aim of this approach was not to investigate the influence of single neurodegenerative risk factors apart from age on FC. However, we intended to increase the probability of the overall study cohort to already present (age-related) neurodegenerative changes influencing FC measurements Noteworthy, only the FC between the left IFG and right SAC was positively correlated with age in our investigation. A generally increased cortical activity measured via fNIRS in older participants during performance of the TMT has already been described by us as well as discussed within the context of a possible compensation mechanism (Blum et al., 2021). For instance, Respino et al. (2020) reported a positive correlation between an elevated regional homogeneity within the dACC as part of the CCN and executive performance in

the context of late-life depression and suggested this to be a possible compensation mechanism, too.

Some limitations of our study should also be mentioned. First, the used neuroimaging method of fNIRS has established itself as a reliable alternative to fMRI in FC studies because it combines an easy clinical integration with ecologically valid conditions (realistic environment, sitting position, task accomplishment, social interaction) as well as relatively high time resolution (Ehlis et al., 2014). But unlike MRI-based technology, fNIRS does not allow to put functional and structural information into relation; this is important to note as on the one hand it is well known that particularly at early stages of a neurodegenerative disease the correlation between cognitive dysfunction and structural gray or white matter changes can by no means be presumed (Dalaker et al., 2009; Dalaker et al., 2010); on the other hand, a relevant cortical atrophy especially in fronto-temporal areas has been described even in elderly with low probability of AD (Fjell et al., 2014). Further, due to its shallow penetration depth of 2-3 cm into the cranial calotte (Haeussinger et al., 2011), only superficial cortical structures are measured, deeper connections to and within white matter structures will not be captured; this might be a disadvantage within the context of dementia entities with relevant subcortical involvement of pathology, e.g. Parkinson's Disease or vascular dementia. Finally, albeit the CCN and DAN have already been shown to be accessible for fNIRS investigation (Rosenbaum, Hilsendegen, et al., 2018), subsequent analysis is also limited to the corresponding pre-defined ROIs. Besides, the spatial specificity of fNIRS compared to fMRI is reduced, which means that measuring FC within a region by correlation techniques can overestimate the real FC, because the individual channels may access overlapping areas. Anyway, the combination of fNIRS measurement with the TMT task has already been shown to be suitable for the investigation of elderly subjects as well as the detection of aging-related differences in resulting cortical activation patterns (Hagen et al., 2014; Hofmann et al., 2021; Rosenbaum, Blum, et al., 2018; Rosenbaum et al., 2016). The TMT allows the integration into many clinical study settings because it is an easy-to-

handle paper-pencil task that offers a natural testing situation and does not provoke any artifacts by activating the mimic musculature by speech.

Next, our subject sample shows an age range of 50 to 85 years as FC changes during later life as well as associated executive deficits were the main focus of the study at hand. So, one could argue that the omission of younger volunteers prevents a complete picture of FC changes over the lifespan, although previous studies have already shown that at least age-related cognitive deficits do not become apparent until about 50 years of age (Oosterman et al., 2010; Salthouse, 2010; Singh-Manoux et al., 2012; Wecker et al., 2005). Accordingly, one might assume that even the concomitant FC changes during task performance are not expected to appear before that age, either. However, Hofmann et al. (2021) observed a significantly reduced neural activity in the right DLPFC also via fNIRS within prodromal Parkinson's Disease patients completing the TMT-A and -B in contrast to the TMT-C, even before differences became evident on the behavioral level.

Finally, as described above, we investigated a sub-cohort of the TREND study collective, which is enriched for other neurodegenerative risk factors apart from age. This is why, we matched the two groups of early and late elders, amongst others, for these factors (table 5). Albeit thus equally in both subgroups, this may bias our results, nevertheless. Therefore, within future studies, it will be important to potentially create a cohort of healthy agers explicitly free from other neurodegenerative risk factors or enriched for only single of these risk factors. Such an approach might help to selectively investigate the influence of other risk factors for neurodegenerative diseases, like RBD, on FC during the prodromal stage.

2.3.5. Conclusion

To the authors' knowledge, this is the first study examining the influence of age on functional connectivity through a comparison of RS and task-related measurements (TMT) using fNIRS.

To sum up, only particular regions of the CCN and DAN were affected by an age-related FC decrease. This finding was observed for the resting-state

measurement and was even more pronounced during execution of the TMT, and it might indicate a specific vulnerability of these areas to ageing and/or early neurodegenerative processes. Further, with the aim of identifying age-related – either physiological or pathological – FC changes as early as possible, these results confirm our hypothesis that measuring these during task conditions is superior to the RS.

Therefore, it will be very important to confirm these promising findings within future studies, under special consideration of multiple task conditions on FC characteristics of the corresponding networks. Especially the application of a dual task situation might be a promising approach to further understand this dynamic and complex interplay. For instance, Beurskens et al. (2014) have already shown within their fNIRS study that especially the combination of a cognitive with a simultaneous motor task can have a relevant impact on neural functionality within older adults. Finally, the constant correlation with behavioral performance will maintain a key role for assessment of potentially successful, neural compensation mechanisms.

3. General discussion

3.1. Discussion of research questions

The present dissertation aimed at the analysis of age-related changes in cortical activation, functional connectivity and cognitive performance. In order to assess age-related cortical restructuring, three studies were conducted to answer a total of eight research questions. In the following section, the research questions formulated in the introduction will be compared with the predefined hypotheses, and the current state of research as well as the results obtained.

- *Research question 1:* Are there differences between the influence of working speed (slow versus medium versus fast) and task complexity (TMT-A vs. TMT-B) on the hemodynamic response?

We already know that both block designs and event-related designs are used in the evaluation of cognitive tasks such as TMT (Hagen et al., 2014; Muller et al., 2014). Furthermore, it can be assumed that when using time-restricted block designs both speed and task complexity positively influence cortical blood oxygenation, which makes the interpretation of the results more difficult. Especially previous time-restricted study designs could not necessarily detect the expected combination of higher hemodynamic responses with increased task complexity (Hagen et al., 2014; Muller et al., 2014).
An fNIRS study by Artemenko et al. (2018) found lower cortical activity in the execution of more complex mathematical tasks compared to simpler calculations. Only after integration of the factor "task complexity" by the number of calculated equations, a positive correlation between a more complex degree of difficulty and a higher hemodynamic response was found (Artemenko et al., 2018).

Our results show increased influences of working speed on the hemodynamic response in the area of bilateral IFG, SAC, and left dlPFC. The correction of the dependent variable for the number of processed items let differential effects

between the TMT subtests become visible. However, no effects on task complexity by distinguishing the influences of TMT-A and TMT-B were observed in the uncorrected data, which is in line with previous findings mentioned above. This discovery was of particular importance, as an older group of subjects was the focus of our research. Especially in older participants, a compensation of cognitive deficits in TMT-B by reduction of the working speed is probable.

- *Research question 2:* Does the use of a time-limited block design level performance differences in TMT?

The use of time-restricted block design can be considered problematic if the performance component is not taken into account by the number of solved items. In this case, it could not be ruled out that impairments, probably particularly noticeable in the more complex task of the TMT-B, may be compensated by a reduction in working speed. The available results show that two methods (correction by regression and correction by ratio calculation (O_2Hb per solved item)) are equally suitable for the representation of the influences of task complexity and working speed. The assumption of the necessity of a correction method for the number of solved items for the representation of inter-individual performance differences is confirmed by our results. Thus, only after the correction for the number of processed items, a differential effect for TMT in SPL was shown.

- *Research question 3:* Are there age differences in TMT performance (number of processed items)?

The TMT performance depends largely on the respective sub-test (TMT-A versus TMT-B). It is believed that the TMT-B requires higher cognitive abilities, an assumption that is consistent with previous findings of more processed items during TMT-A compared to the more demanding TMT-B (Gaudino et al., 1995; Muller et al., 2014; Seo et al., 2006). With regard to age effects, previous studies agree on the assumption of more items solved during TMT in younger participants

compared to older participants (Goul & Brown, 1970; Kennedy, 1981; Muller et al., 2014; Rodewald et al., 2012; Seo et al., 2006; Tombaugh, 2004). A reduced working speed in older adults is particularly evident in the more complex TMT-B (Goul & Brown, 1970; Hamdan & Hamdan, 2009; Rasmusson et al., 1998; Rodewald et al., 2012).

As hypothesized, our results were able to confirm the previous research findings to the effect that fewer items could be solved during TMT-B in comparison to TMT-A regardless of age. Additionally, older subjects showed a reduced working speed during TMT in general compared to the younger subjects. We explain these findings as a representation of the underlying increased cognitive demands in TMT-B by, for example, the required task-switching. It seems obvious that a behavioral age deficit will first appear during more complex tasks.

- *Research question 4:* How do age groups differ concerning the cortical blood oxygenation during task performance (time-controlled)?

A widespread thesis assumes the compensation of age-related limitations in cognitive tasks by increasing the cortical activity, and/or recruiting additional cortical areas. In various studies, this assumption of performance maintenance by overactive cortical regions (C. L. Grady, 2008; Rossi et al., 2004; Spreng et al., 2010), or an increasingly bilateral activation with age (Cabeza, 2002) seemed to be confirmed.

In line with the previously mentioned studies, our results presented a decisive age-effect in areas of the cognitive control network (CCN), which was characterized by higher blood oxygenation in older subjects than in younger subjects. According to our previously formulated hypothesis, we interpreted the increase in activity as a compensatory mechanism for the cognitive decline of the elderly. A frequently discussed mechanism underlying cognitive impairment in old age is the atrophy of the white and grey matter (Courchesne et al., 2000; Pfefferbaum et al., 1994; Walhovd et al., 2005). Accordingly, tissue reduction

leads to loss of performance in cognitive processes unless endogenous mechanisms such as recruitment and over-activation compensate for them.

- *Research question 5:* How do the age groups differ with regard to cortical blood oxygenation per solved item (item-controlled)?

The results of our first study confirmed the validity of time-restricted block designs when data will be supplemented with the calculated ratio variable (O_2Hb concentration per item). Thus, enabled an analysis comparable to an event-related design. Contrary to the time-controlled results described above, after correction of the data for the number of solved items, effects for age became no longer evident. As opposed to the pre-formulated hypothesis, the item-correction eliminated the age effect, although the age groups differed significantly in terms of behavioral performance data, and there was a negative linear relationship between age and number of completed items. A more detailed investigation explained this effect as a consequence of a negative quadratic relationship between age and O_2Hb levels in left DLPFC and right SAC. The effect is characterized by an increase in O_2Hb values until the turning point at about 65-70 years and a subsequent decrease.

- *Research question 6:* Can age-related compensation mechanisms be assumed?

The increase of frontal activity and stronger lateralization with increasing age has been reported many times (Cabeza, 2002; Cappell et al., 2010; C. L. Grady et al., 2005; Muller et al., 2014; D. C. Park et al., 2001; P. Reuter-Lorenz, 2002; P. A. Reuter-Lorenz et al., 2000; P. A. Reuter-Lorenz & Lustig, 2005; Rossi et al., 2004).

The suspicion of age-dependent mechanisms to compensate for impairments is corroborated by the research results of the second study. At an advanced age, a decrease in TMT performance and a contemporaneous increase in cortical blood

oxygenation was observed, which suggests a cortical over-activity or recruitment of additional cortical resources. Moreover, the contrast in O_2Hb levels between the age groups, which is characterized by a greater increase in blood oxygenation for the more complex TMT-B within the older subjects, should be emphasized.
Nevertheless, the interpretation of these findings as compensation mechanisms must be viewed critically, since there are strong interdependencies between the causes and influences of compensatory and pathological effects. Unfortunately, an unambiguous classification of the observed effects is not possible with our methodology. And even in the history of research to date, views on the concept of compensation vary greatly. For example, age-related additional bilateral recruitment of cortical resources was interpreted as compensation both if it was accompanied by increased (Springer et al., 2005) or reduced (Steffener et al., 2009) performance. The latter phenomenon was called “attempted” and “unsuccessful” compensation (Cabeza & Dennis, 2013). The term describes the case when cognitive demand is unsuccessfully compensated by increased activity. Thus, results in a worse performance despite increased activity.
Furthermore, the compensation hypothesis has to be questioned by the results of our item-corrected analysis, as the consideration of individual performance erased the age effect. Alternatively to the compensation hypotheses, increased activity in elderly was interpreted as an inability to suppress cortical regions that do not contribute to cortical processing of task demands. Reductions in specificity and efficiency were also discussed as causes of increased O_2Hb levels (Alexa M. Morcom & Henson, 2018). Another explanation for inter-individual differences in performance is based on pre-existing neuronal reserve capacities. These cognitive resources vary individually, and in the susceptibility to age influences (Stern, 2009).

- *Research question 7:* Does functional connectivity (FC) differ from older and younger participants during the resting state measurement and task performance? If so, what ROI would be affected?

Based on previous studies, we already know of age effects on functional connectivity. However, in contrast to our study concept, these mostly analyzed resting state functional connectivity (Betzel et al., 2014; Chan et al., 2014; Ferreira & Busatto, 2013; Schlee et al., 2012; Siman-Tov et al., 2017; Zonneveld et al., 2019). In several studies, age effects were differentiated according to intranetwork FC (within) and internetwork FC (between) (Chan et al., 2014; C. Grady et al., 2016; Spreng et al., 2016). While older participants had a lower FC within the networks, and a higher FC between the networks, the ratios were reversed for younger subjects (Chan et al., 2014; C. Grady et al., 2016; Spreng et al., 2016).

According to our forecasts, we found various effects for age in specific ROIs. Whereas several ROIs showed a negative correlation between age and FC, age correlated positively with FC between lIFG and rSAC. Especially in the FC within the left IFG, the elderly participants showed a reduced FC both at resting state and during TMT performance.

- *Research question 8:* Are age effects more visible during task performance than during resting state measurements?

With regard to the different cognitive states (resting state versus TMT-C versus TMT-A versus TMT-B), significant differences in functional connectivity were found. Further, our pre-defined hypothesis that FC is a particularly sensitive marker for the detection of age-dependent cerebral reorganizations was confirmed. For explanation, it should be added that our hypothesis was based on the assumption that age effects would be amplified by the increased performance requirements in task conditions compared to resting state measurements. This assumption could not be supported by the results, as regarding age effects, lower FC in older than in younger subjects was seen in both, the TMT and the resting state measurement. But the main effect for age was not highlighted when participants were performing TMT. Nevertheless, regardless of age, between networks heightened FC values during TMT execution compared to resting state

measurement became apparent. At the same time, the within network FC was higher at rest than during the task performance.

3.2. Limitations

In addition to the numerous important findings from our studies, a few limitations must also be addressed. As already mentioned in the different studies, concerning the used neuroimaging method these are the restricted spatial resolution of fNIRS measurements, the limited coverage of the brain area by fNIRS optodes, and the susceptibility to artifacts due to movements of the masticatory and mimic musculature. The latter problem has necessitated the use of strict correction methods. Another critical issue is the calculation of FC using theory-driven pre-defined networks, which cannot reflect the underlying structural and functional networks exactly.

However, apart from the limitations already discussed, the interpretation of the results must also be critically discussed. In the presented studies it remains unclear whether the obtained results in activity and FC in fact are compensation effects or pathological changes of the aging brain. Since our subjects from studies two and three included a sample of elderly healthy subjects and elderly subjects with risk factors for neuropathologies, the influence of degenerative processes cannot be clearly defined. Our concept was based on the comparison of two age groups, the individual risk factors represented in our group of participants such as RBD, Hyposmia and aMCI were not considered separately. However, since the groups were matched according to neurodegenerative risk factors, no distortion of the results must be assumed. It should be added that no genuine neurodegeneration markers were determined.

Nevertheless, an unequivocal differentiation of the influences of compensatory and pathological effects is difficult because of strong interactions and interdependencies. In fact, both the endogenous attempt to compensate for neuropathologies and the pathology itself can be interpreted as compensation for environmental influences. Consequently, heightened activation in the more demanding task of TMT-B within the older population may also be due to

increased stress levels experienced by the older population when participating in cognitively complex tasks.

Another point of criticism arises from the design of the TREND study, from which the subjects of studies two and three originated. The data presented came from the second follow-up of the TREND study, which is why exercise effects from the first two test runs are possible. However, the experimental follow-ups are two years apart. For TMT, no more exercise effects could be demonstrated with repetitions more than 12 months apart (Basso et al., 1999). Remarkably, the participants of the TREND-study showed an interesting effect: Throughout the entire study, men had a higher level of education than women, an effect that increases with the age of the participants. Consequently, after age-matching, no simultaneous matching of the groups by gender and education was possible. To account for this fact, the factors gender and education were examined as covariates. However, their influences were negligible.

Another conspicuous feature within the cohort was the difference in the intake of anticoagulants. While, as expected, the older participants consumed more anticoagulants than the younger ones, the same effect was also observed between the gender groups. Thus, men took significantly more blood thinners than women. Considering the consumption of anticoagulants as a marker for underlying vascular diseases, a connection to neurodegenerative diseases cannot be excluded.

3.3. Remaining questions and future outlooks

Some questions and future perspectives have already been addressed in the previous chapters. The following lines are intended to provide a summary of the research questions remaining from the presented studies.

The central question was already introduced in the chapter “limitations”: Are changes in cortical activation and functional connectivity due to compensatory or psychopathological mechanisms? It will be content of future research to focus on the differentiation and disentanglement of compensatory and neurodegenerative effects in longitudinal studies. Furthermore, the transformation of the generated

findings into medically feasible treatment strategies remains a topic for further research. For example, with the help of regular neurophysiological examinations of brain activity, physiological ageing processes could be distinguished from neuropathological changes. Consequently, the latter can be detected and treated at the earliest possible point in time. At this point, in addition to our study design of two age groups (younger subjects: age 53-65 years; older subjects: age 66-84 years), a study of multiple age groups for the fine differentiation of age progressions would be interesting.

4. Summary and conclusions

Age, as a central risk factor for neurodegenerative diseases, was the focus of the studies presented. The investigation of neurophysiological brain organization by measuring cortical activation and functional connectivity is regarded as a promising predictor for the classification of physiological and pathological aging processes. Our experimental design is based on the hypothesis that age-related limitations would first manifest themselves in complex cognitive challenges. For this reason, we focused on comparing measurements of cortex activation and functional connectivity at rest and during the execution of cognitive tests. A special interest was also the relationship between the neurophysiological measurement data obtained and the behavioral data of cognitive performance.

The dissertation comprises a total of three studies that agree in the methodology applied. In all studies, functional near-infrared spectroscopy (fNIRS) was used to measure cortical blood oxygenation. The cognitive parameters of executive functions were examined using the modified Trail Making Test (TMT), consisting of TMT-A, TMT-B, and TMT-C, from the CERAD-Plus test battery. Study one included a total of 20 subjects (mean age 27 years). Study two (250 participants, mean age 66 years) and study three (218 participants, mean age 66 years) comprised a sample of elderly participants from the TREND-study.

While the first study was dedicated to establishing a new correction method for the number of processed items in TMT as a performance variable to improve the validity of block designs, studies two and three focused on the overarching question of ageing processes.

In summary, study one showed that both task complexity (TMT-A versus TMT-B) and processing speed (slow versus moderate versus fast) influence cortical oxygen saturation, requiring the integration of a correction method. As a consequence, we were able to prove the proposed ratio calculation (O_2Hb per processed item) as valid.

The correction method introduced was already applied in the second study. The second investigation addressed the behavioral and neurophysiological age differences in the performance of TMT by measuring cortical activation. Besides

a reduction of the working speed, higher blood oxygenation within the older participants (age 66-84 years) compared to the younger group (age 53-65 years) was observed. Regarding the three TMT conditions, higher O_2Hb levels were found in TMT-A and TMT-B compared to TMT-C, as well as in TMT-B compared to TMT-A.

The third study included a comparison of the FC of two age groups during resting state measurement and TMT execution. Interestingly, there were no major age effects with respect to the different mental states (resting state versus TMT-C versus TMT-A versus TMT-B). Nevertheless, age effects were observed in individual ROIs. In addition, interesting differences in FC within and between regions emerged. While the between regions FC was lower at rest than during TMT completion, the within regions FC was characterized by higher values at rest than during TMT.

To sum it up, our studies showed that TMT and fNIRS are suitable non-invasive and ecologically valid methods for the detection of cerebral ageing processes. Our results allow the suspicion of age-related compensatory mechanisms, which needs to be substantiated in further studies.

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6. Supplemental Material

6.1. Tables

Table 8: Allocation channels and corresponding brain areas of the fNIRS probesets used in study 2 and study 3.

Brain area	Probeset: left frontal	Probeset: right frontal
left dorsolateral prefrontal cortex	4, 7, 8, 9, 10, 11, 12	
right dorsolateral prefrontal cortex		13, 18, 20, 21, 23, 24
left inferior frontal gyrus	1, 3, 6	
right inferior frontal gyrus		14, 16, 19
pars opercularis, Broca's area	8	17
premotor and supplementary motor cortex		22
frontopolar area	2, 5	15
	Probeset: parietal	
left somatosensory association cortex	26, 29, 31	
right somatosensory association cortex	33, 35, 36, 38	
supramarginal gyrus part of Wernicke's area	37	
primary motor cortex	27, 32	
premotor and supplementary motor cortex	30	
primary somatosensory cortex	25, 28, 34	

Table 9: Supplemental table of study 3: Level of significance for different ROI activation patterns in fNIRS according to the task condition: Niveau 1 = TMT-C, Niveau 2 = TMT-A, Niveau 3 = TMT-B, Niveau 4 = resting state, DLPFC = dorso-lateral prefrontal cortex, IFG = inferior frontal gyrus, SAC = sensory association cortex.

Source	Measure	TMT	Sum of squares	df	Mean of squares	F	Level of significance	Partial Eta-square
TMT	ldlpfc_lifg	Niveau 1 vs. Niveau 4	3,732	1	3,732	17,460	,000	,083
		Niveau 2 vs. Niveau 4	4,304	1	4,304	18,846	,000	,089
		Niveau 3 vs. Niveau 4	2,226	1	2,226	12,586	,000	,061
	ldlpfc_rifg	Niveau 1 vs. Niveau 4	4,537	1	4,537	24,515	,000	,112
		Niveau 2 vs. Niveau 4	3,580	1	3,580	18,963	,000	,089
		Niveau 3 vs. Niveau 4	3,354	1	3,354	19,048	,000	,089
	ldlpfc_rdlpfc	Niveau 1 vs. Niveau 4	1,223	1	1,223	9,692	,002	,048
		Niveau 2 vs. Niveau 4	,194	1	,194	1,280	,259	,007
		Niveau 3 vs. Niveau 4	,094	1	,094	,754	,386	,004
	ldlpfc_rsac	Niveau 1 vs. Niveau 4	,014	1	,014	,102	,750	,001
		Niveau 2 vs. Niveau 4	,009	1	,009	,059	,808	,000
		Niveau 3 vs. Niveau 4	,114	1	,114	1,096	,297	,006
	ldlpfc_lsac	Niveau 1 vs. Niveau 4	,002	1	,002	,015	,902	,000
		Niveau 2 vs. Niveau 4	,087	1	,087	,546	,461	,003
		Niveau 3 vs. Niveau 4	,153	1	,153	1,386	,241	,007
	ldlpfc_within	Niveau 1 vs. Niveau 4	1,342	1	1,342	8,572	,004	,042
		Niveau 2 vs. Niveau 4	2,311	1	2,311	14,265	,000	,068
		Niveau 3 vs. Niveau 4	5,219	1	5,219	41,317	,000	,176
	rdlpfc_lifg	Niveau 1 vs. Niveau 4	3,049	1	3,049	18,409	,000	,087
		Niveau 2 vs. Niveau 4	1,782	1	1,782	9,857	,002	,048
		Niveau 3 vs. Niveau 4	3,195	1	3,195	21,360	,000	,099
	rdlpfc_rifg	Niveau 1 vs. Niveau 4	3,903	1	3,903	22,454	,000	,104
		Niveau 2 vs. Niveau 4	4,169	1	4,169	19,747	,000	,092
		Niveau 3 vs. Niveau 4	5,489	1	5,489	33,246	,000	,146
	rdlpfc_rsac	Niveau 1 vs. Niveau 4	,933	1	,933	6,660	,011	,033
		Niveau 2 vs. Niveau 4	,943	1	,943	6,304	,013	,031
		Niveau 3 vs. Niveau 4	,595	1	,595	5,507	,020	,028
	rdlpfc_lsac	Niveau 1 vs. Niveau 4	,411	1	,411	2,822	,095	,014
		Niveau 2 vs. Niveau 4	,530	1	,530	3,434	,065	,017
		Niveau 3 vs. Niveau 4	,321	1	,321	2,743	,099	,014
	rdlpfc_within	Niveau 1 vs. Niveau 4	,563	1	,563	4,686	,032	,024
		Niveau 2 vs. Niveau 4	,394	1	,394	3,032	,083	,015
		Niveau 3 vs. Niveau 4	,735	1	,735	6,225	,013	,031
	lifg_rifg	Niveau 1 vs. Niveau 4	,011	1	,011	,054	,816	,000
		Niveau 2 vs. Niveau 4	,098	1	,098	,455	,501	,002
		Niveau 3 vs. Niveau 4	,141	1	,141	,850	,358	,004
	lifg_rsac	Niveau 1 vs. Niveau 4	1,401	1	1,401	7,357	,007	,037
		Niveau 2 vs. Niveau 4	,290	1	,290	1,438	,232	,007
		Niveau 3 vs. Niveau 4	1,812	1	1,812	10,251	,002	,050
	lifg_lsac	Niveau 1 vs. Niveau 4	1,780	1	1,780	8,425	,004	,042
		Niveau 2 vs. Niveau 4	,195	1	,195	,980	,323	,005
		Niveau 3 vs. Niveau 4	1,558	1	1,558	8,422	,004	,042

	lifg_within	Niveau 1 vs. Niveau 4	1,367	1	1,367	5,273	,023	,026
		Niveau 2 vs. Niveau 4	1,458	1	1,458	5,804	,017	,029
		Niveau 3 vs. Niveau 4	,382	1	,382	1,620	,205	,008
	rifg_rsac	Niveau 1 vs. Niveau 4	2,209	1	2,209	12,980	,000	,063
		Niveau 2 vs. Niveau 4	1,451	1	1,451	7,007	,009	,035
		Niveau 3 vs. Niveau 4	4,540	1	4,540	28,412	,000	,128
	rifg_lsac	Niveau 1 vs. Niveau 4	1,821	1	1,821	9,112	,003	,045
		Niveau 2 vs. Niveau 4	1,253	1	1,253	5,891	,016	,029
		Niveau 3 vs. Niveau 4	4,242	1	4,242	24,472	,000	,112
	rifg_within	Niveau 1 vs. Niveau 4	,391	1	,391	1,520	,219	,008
		Niveau 2 vs. Niveau 4	,003	1	,003	,014	,905	,000
		Niveau 3 vs. Niveau 4	,265	1	,265	1,095	,297	,006
	rsac_lsac	Niveau 1 vs. Niveau 4	,721	1	,721	4,124	,044	,021
		Niveau 2 vs. Niveau 4	,678	1	,678	4,255	,040	,021
		Niveau 3 vs. Niveau 4	,058	1	,058	,419	,518	,002
	rsac_within	Niveau 1 vs. Niveau 4	,536	1	,536	3,364	,068	,017
		Niveau 2 vs. Niveau 4	,360	1	,360	2,521	,114	,013
		Niveau 3 vs. Niveau 4	,879	1	,879	6,559	,011	,033
	lsac_within	Niveau 1 vs. Niveau 4	1,188	1	1,188	7,343	,007	,036
		Niveau 2 vs. Niveau 4	1,824	1	1,824	11,359	,001	,055
		Niveau 3 vs. Niveau 4	2,915	1	2,915	19,097	,000	,090
TMT * age	ldlpfc_lifg	Niveau 1 vs. Niveau 4	,015	1	,015	,070	,791	,000
		Niveau 2 vs. Niveau 4	,058	1	,058	,255	,614	,001
		Niveau 3 vs. Niveau 4	,112	1	,112	,632	,428	,003
	ldlpfc_rifg	Niveau 1 vs. Niveau 4	,047	1	,047	,255	,614	,001
		Niveau 2 vs. Niveau 4	,364	1	,364	1,930	,166	,010
		Niveau 3 vs. Niveau 4	,002	1	,002	,011	,916	,000
	ldlpfc_rdlpfc	Niveau 1 vs. Niveau 4	,013	1	,013	,104	,748	,001
		Niveau 2 vs. Niveau 4	,000	1	,000	,001	,969	,000
		Niveau 3 vs. Niveau 4	6,177E-6	1	6,177E-6	,000	,994	,000
	ldlpfc_rsac	Niveau 1 vs. Niveau 4	,099	1	,099	,719	,398	,004
		Niveau 2 vs. Niveau 4	,001	1	,001	,008	,929	,000
		Niveau 3 vs. Niveau 4	,005	1	,005	,052	,820	,000
	ldlpfc_lsac	Niveau 1 vs. Niveau 4	,356	1	,356	2,299	,131	,012
		Niveau 2 vs. Niveau 4	,008	1	,008	,051	,821	,000
		Niveau 3 vs. Niveau 4	,001	1	,001	,005	,942	,000
	ldlpfc_within	Niveau 1 vs. Niveau 4	,092	1	,092	,590	,443	,003
		Niveau 2 vs. Niveau 4	,082	1	,082	,506	,478	,003
		Niveau 3 vs. Niveau 4	,121	1	,121	,959	,329	,005
	rdlpfc_lifg	Niveau 1 vs. Niveau 4	,141	1	,141	,853	,357	,004
		Niveau 2 vs. Niveau 4	,241	1	,241	1,335	,249	,007
		Niveau 3 vs. Niveau 4	,231	1	,231	1,542	,216	,008
	rdlpfc_rifg	Niveau 1 vs. Niveau 4	,028	1	,028	,162	,688	,001
		Niveau 2 vs. Niveau 4	,017	1	,017	,080	,777	,000
		Niveau 3 vs. Niveau 4	,005	1	,005	,031	,861	,000
	rdlpfc_rsac	Niveau 1 vs. Niveau 4	,525	1	,525	3,745	,054	,019
		Niveau 2 vs. Niveau 4	,058	1	,058	,389	,534	,002
		Niveau 3 vs. Niveau 4	,318	1	,318	2,946	,088	,015
	rdlpfc_lsac	Niveau 1 vs. Niveau 4	,268	1	,268	1,841	,176	,009
		Niveau 2 vs. Niveau 4	,112	1	,112	,727	,395	,004
		Niveau 3 vs. Niveau 4	,628	1	,628	5,358	,022	,027

	rdlpfc_within	Niveau 1 vs. Niveau 4	,275	1	,275	2,289	,132	,012
		Niveau 2 vs. Niveau 4	,172	1	,172	1,323	,252	,007
		Niveau 3 vs. Niveau 4	,041	1	,041	,344	,558	,002
	lifg_rifg	Niveau 1 vs. Niveau 4	,136	1	,136	,659	,418	,003
		Niveau 2 vs. Niveau 4	,130	1	,130	,607	,437	,003
		Niveau 3 vs. Niveau 4	,053	1	,053	,319	,573	,002
	lifg_rsac	Niveau 1 vs. Niveau 4	,399	1	,399	2,097	,149	,011
		Niveau 2 vs. Niveau 4	,001	1	,001	,005	,944	,000
		Niveau 3 vs. Niveau 4	,217	1	,217	1,225	,270	,006
	lifg_lsac	Niveau 1 vs. Niveau 4	,261	1	,261	1,237	,267	,006
		Niveau 2 vs. Niveau 4	,007	1	,007	,037	,848	,000
		Niveau 3 vs. Niveau 4	,680	1	,680	3,676	,057	,019
	lifg_within	Niveau 1 vs. Niveau 4	,217	1	,217	,837	,361	,004
		Niveau 2 vs. Niveau 4	,922	1	,922	3,672	,057	,019
		Niveau 3 vs. Niveau 4	,399	1	,399	1,693	,195	,009
	rifg_rsac	Niveau 1 vs. Niveau 4	,154	1	,154	,906	,342	,005
		Niveau 2 vs. Niveau 4	,169	1	,169	,815	,368	,004
		Niveau 3 vs. Niveau 4	,016	1	,016	,102	,749	,001
	rifg_lsac	Niveau 1 vs. Niveau 4	,002	1	,002	,012	,912	,000
		Niveau 2 vs. Niveau 4	,311	1	,311	1,463	,228	,007
		Niveau 3 vs. Niveau 4	,199	1	,199	1,150	,285	,006
	rifg_within	Niveau 1 vs. Niveau 4	,039	1	,039	,153	,696	,001
		Niveau 2 vs. Niveau 4	,078	1	,078	,353	,553	,002
		Niveau 3 vs. Niveau 4	,004	1	,004	,017	,896	,000
	rsac_lsac	Niveau 1 vs. Niveau 4	,432	1	,432	2,472	,118	,013
		Niveau 2 vs. Niveau 4	,126	1	,126	,788	,376	,004
		Niveau 3 vs. Niveau 4	,386	1	,386	2,768	,098	,014
	rsac_within	Niveau 1 vs. Niveau 4	,281	1	,281	1,766	,185	,009
		Niveau 2 vs. Niveau 4	,201	1	,201	1,411	,236	,007
		Niveau 3 vs. Niveau 4	,421	1	,421	3,140	,078	,016
	lsac_within	Niveau 1 vs. Niveau 4	,537	1	,537	3,321	,070	,017
		Niveau 2 vs. Niveau 4	,366	1	,366	2,282	,132	,012
		Niveau 3 vs. Niveau 4	,196	1	,196	1,281	,259	,007
Error (TMT)	ldlpfc_lifg	Niveau 1 vs. Niveau 4	41,472	194	,214			
		Niveau 2 vs. Niveau 4	44,302	194	,228			
		Niveau 3 vs. Niveau 4	34,305	194	,177			
	ldlpfc_rifg	Niveau 1 vs. Niveau 4	35,901	194	,185			
		Niveau 2 vs. Niveau 4	36,622	194	,189			
		Niveau 3 vs. Niveau 4	34,162	194	,176			
	ldlpfc_rdlpfc	Niveau 1 vs. Niveau 4	24,484	194	,126			
		Niveau 2 vs. Niveau 4	29,350	194	,151			
		Niveau 3 vs. Niveau 4	24,174	194	,125			
	ldlpfc_rsac	Niveau 1 vs. Niveau 4	26,660	194	,137			
		Niveau 2 vs. Niveau 4	30,278	194	,156			
		Niveau 3 vs. Niveau 4	20,252	194	,104			
	ldlpfc_lsac	Niveau 1 vs. Niveau 4	30,028	194	,155			
		Niveau 2 vs. Niveau 4	30,939	194	,159			
		Niveau 3 vs. Niveau 4	21,395	194	,110			
	ldlpfc_within	Niveau 1 vs. Niveau 4	30,375	194	,157			
		Niveau 2 vs. Niveau 4	31,434	194	,162			
		Niveau 3 vs. Niveau 4	24,504	194	,126			

	rdlpfc_lifg	Niveau 1 vs. Niveau 4	32,130	194	,166
		Niveau 2 vs. Niveau 4	35,076	194	,181
		Niveau 3 vs. Niveau 4	29,016	194	,150
	rdlpfc_rifg	Niveau 1 vs. Niveau 4	33,719	194	,174
		Niveau 2 vs. Niveau 4	40,960	194	,211
		Niveau 3 vs. Niveau 4	32,028	194	,165
	rdlpfc_rsac	Niveau 1 vs. Niveau 4	27,178	194	,140
		Niveau 2 vs. Niveau 4	29,023	194	,150
		Niveau 3 vs. Niveau 4	20,972	194	,108
	rdlpfc_lsac	Niveau 1 vs. Niveau 4	28,241	194	,146
		Niveau 2 vs. Niveau 4	29,962	194	,154
		Niveau 3 vs. Niveau 4	22,731	194	,117
	rdlpfc_within	Niveau 1 vs. Niveau 4	23,289	194	,120
		Niveau 2 vs. Niveau 4	25,194	194	,130
		Niveau 3 vs. Niveau 4	22,908	194	,118
	lifg_rifg	Niveau 1 vs. Niveau 4	39,947	194	,206
		Niveau 2 vs. Niveau 4	41,597	194	,214
		Niveau 3 vs. Niveau 4	32,254	194	,166
	lifg_rsac	Niveau 1 vs. Niveau 4	36,944	194	,190
		Niveau 2 vs. Niveau 4	39,120	194	,202
		Niveau 3 vs. Niveau 4	34,296	194	,177
	lifg_lsac	Niveau 1 vs. Niveau 4	40,978	194	,211
		Niveau 2 vs. Niveau 4	38,506	194	,198
		Niveau 3 vs. Niveau 4	35,890	194	,185
	lifg_within	Niveau 1 vs. Niveau 4	50,292	194	,259
		Niveau 2 vs. Niveau 4	48,741	194	,251
		Niveau 3 vs. Niveau 4	45,724	194	,236
	rifg_rsac	Niveau 1 vs. Niveau 4	33,018	194	,170
		Niveau 2 vs. Niveau 4	40,169	194	,207
		Niveau 3 vs. Niveau 4	30,997	194	,160
	rifg_lsac	Niveau 1 vs. Niveau 4	38,763	194	,200
		Niveau 2 vs. Niveau 4	41,247	194	,213
		Niveau 3 vs. Niveau 4	33,629	194	,173
	rifg_within	Niveau 1 vs. Niveau 4	49,866	194	,257
		Niveau 2 vs. Niveau 4	42,829	194	,221
		Niveau 3 vs. Niveau 4	46,922	194	,242
	rsac_lsac	Niveau 1 vs. Niveau 4	33,915	194	,175
		Niveau 2 vs. Niveau 4	30,910	194	,159
		Niveau 3 vs. Niveau 4	27,089	194	,140
	rsac_within	Niveau 1 vs. Niveau 4	30,901	194	,159
		Niveau 2 vs. Niveau 4	27,706	194	,143
		Niveau 3 vs. Niveau 4	26,007	194	,134
	lsac_within	Niveau 1 vs. Niveau 4	31,392	194	,162
		Niveau 2 vs. Niveau 4	31,143	194	,161
		Niveau 3 vs. Niveau 4	29,616	194	,153

6.2. Figures

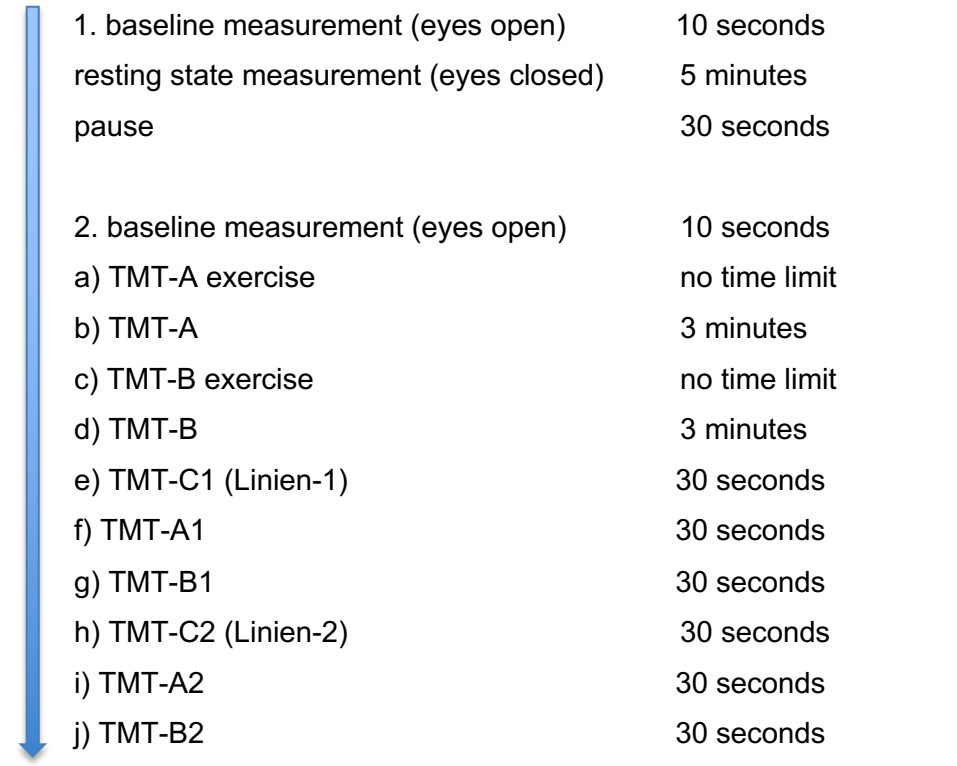


Figure 14: TMT paradigm of study 2 and study 3 according to the TREND-study.

Probanden-ID: ☐☐☐☐ Datum der Untersuchung: __ __ . __ __ . __ __ __ __

NIRS / Ruhemessung & TMT (CERAD)

Schicht: __ Uhrzeit: __ __ : __ __ Position: ___

Untersucher (Kürzel): ___ ___ Unterschrift: _____

Kopfumfang:_____cm Inion-Nasion:_____cm T3-T4 Abstand:_____cm

NIRS Ruhemessung: Anmerkungen / Artefakte (Zeitpunkt, Sek.):

_____

NIRS TMT / CERAD:

TMT A: Zeit _____ Fehler _____ Anzahl/Fehler (30 s) _____ Anm._____

TMT B: Zeit _____ Fehler _____ Anzahl/Fehler (30 s) _____ Anm. _____

NIRS-TMT (30s)

Linien 1: Anzahl _____ Fehler _____ Anm. _____

TMT A1: Anzahl _____ Fehler _____ Anm. _____

TMT B1: Anzahl _____ Fehler _____ Anm. _____

Linien 2: Anzahl _____ Fehler _____ Anm. _____

TMT A2: Anzahl _____ Fehler _____ Anm. _____

TMT B2: Anzahl _____ Fehler _____ Anm. _____

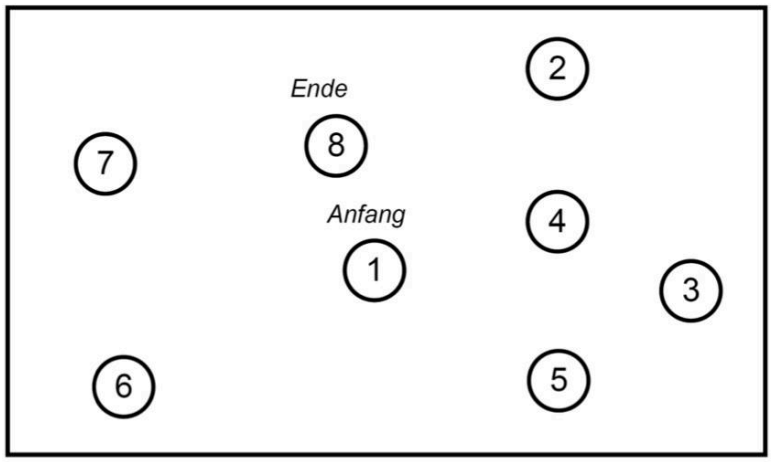
Kommentar NIRS CERAD-TMT:_____

Kommentar NIRS TMT: _____

Probanden-ID: ☐☐☐☐ Datum der Untersuchung: __ __ . __ __ . __ __ __ __

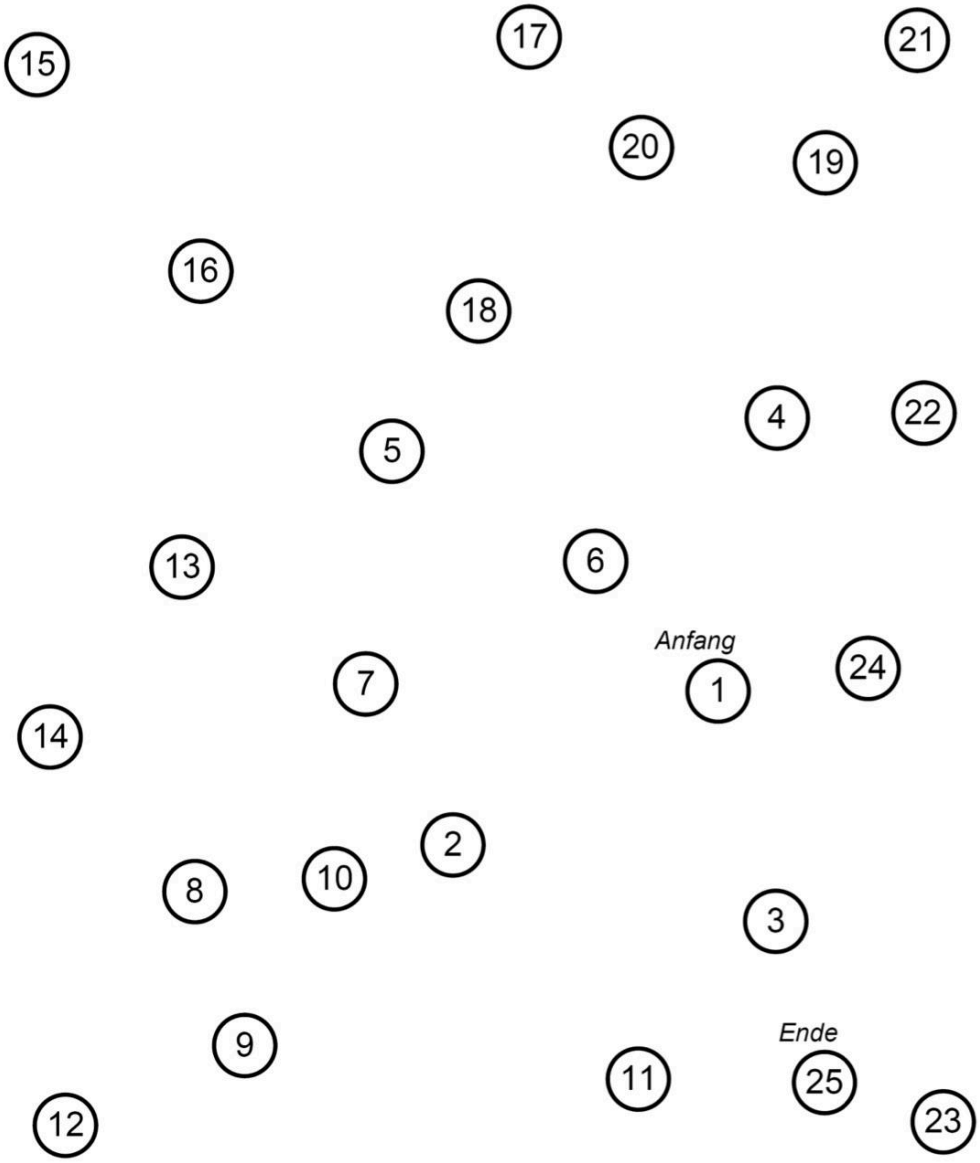
Trail Making Test A:

Übungsbeispiel



Probanden-ID: ☐☐☐☐ Datum der Untersuchung: __ __ . __ __ . __ __ __ __

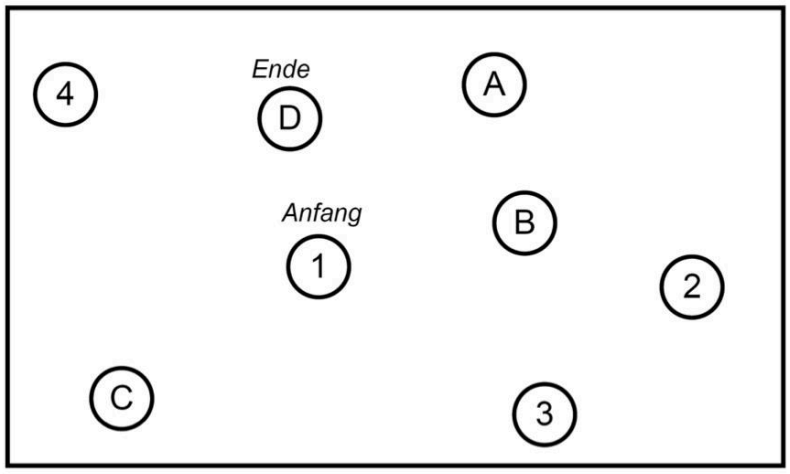
TMT A



Probanden-ID: ☐☐☐☐ Datum der Untersuchung: __ __ . __ __ . __ __ __ __

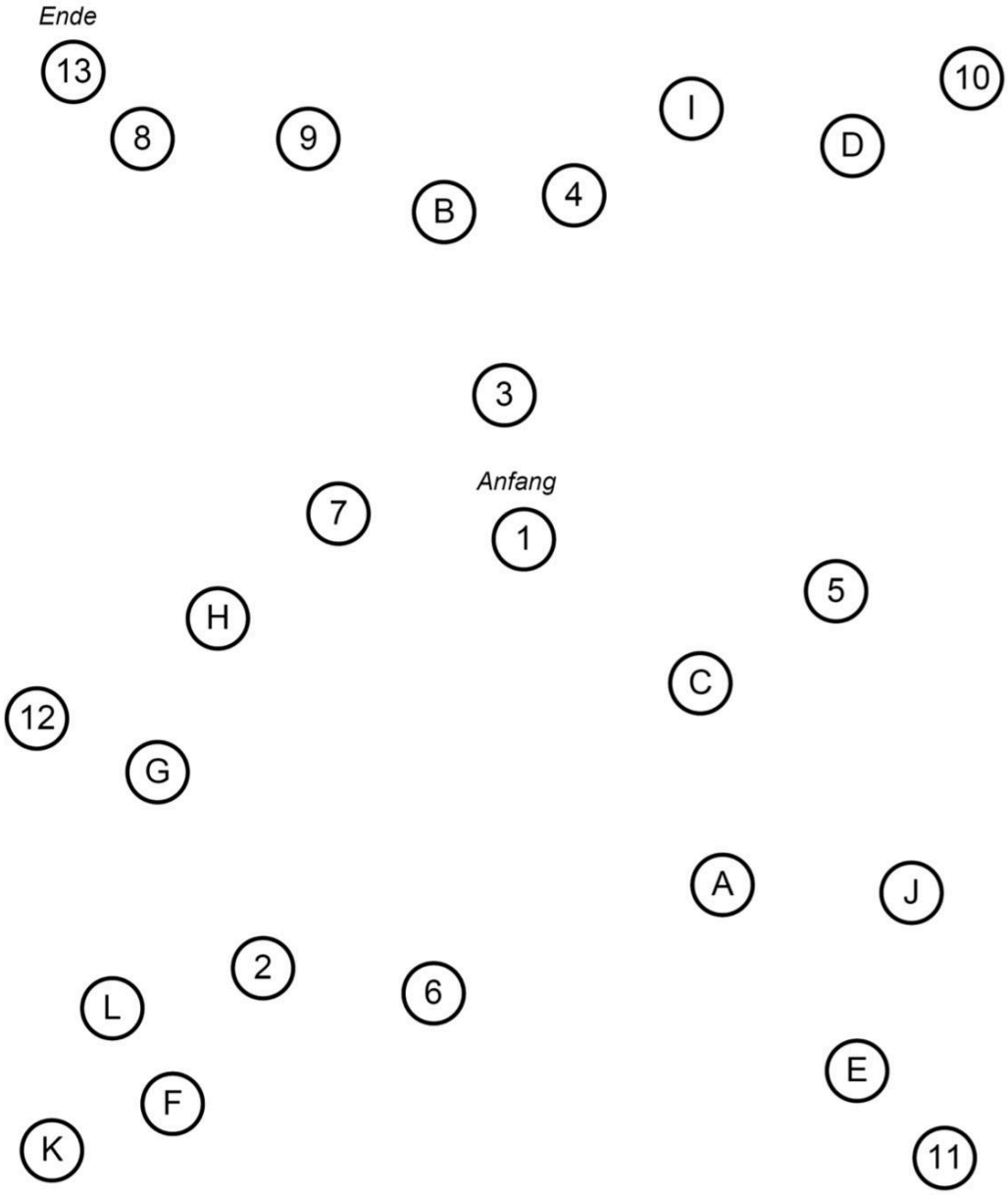
Trail Making Test B

Übungsbeispiel



Probanden-ID: ☐☐☐☐ Datum der Untersuchung: __ __ . __ __ . __ __ __ __

TMT B



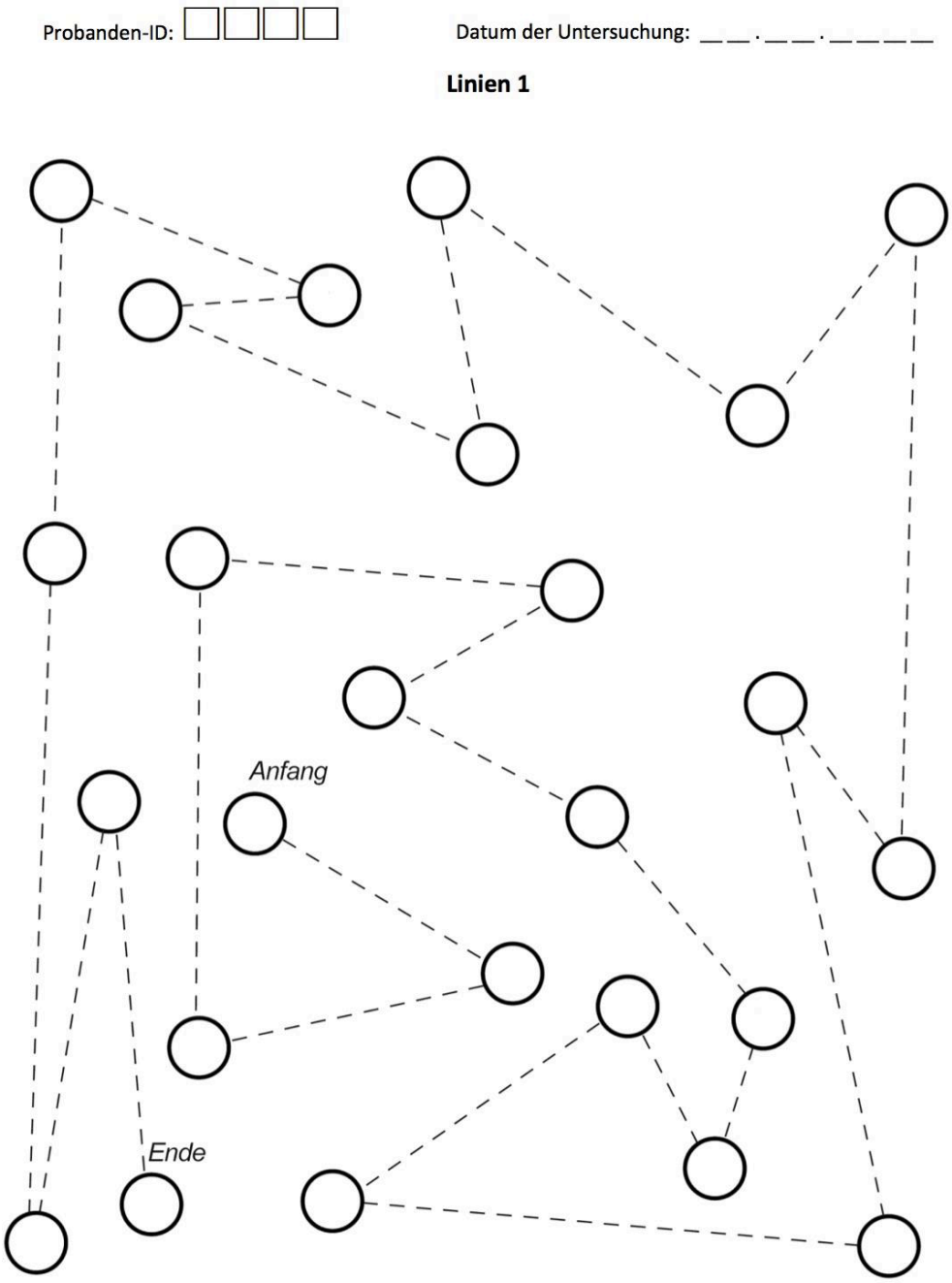


Figure 15, figure 16, figure 17, figure 18, figure 19, figure 20: TMT worksheets according to the TREND-study (study 2 and study 3).

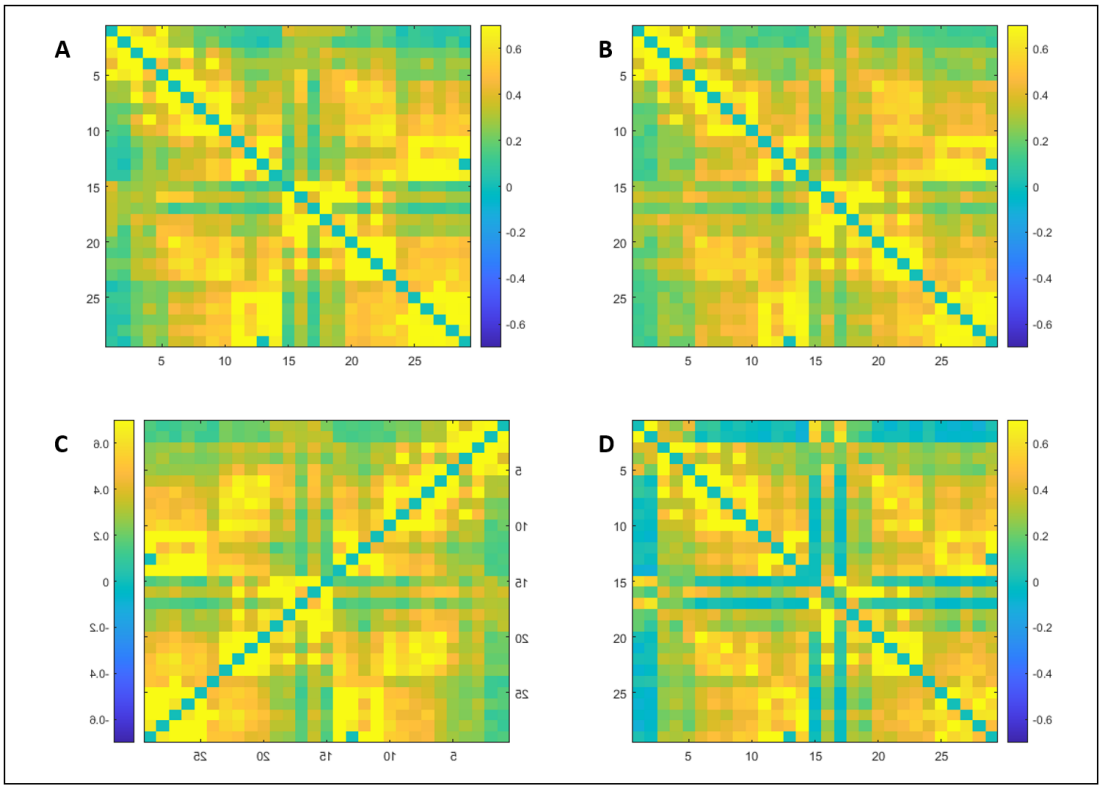


Figure 21: Supplemental figure of study 3: Correlation matrices across all the regions investigated. A: TMT-A, B: TMT-B, C: TMT-C, D: resting state. The different ROIs are indicated via the following rows/columns: 1/4: lIFG, 5/11: lDLPFC, 12/15: lSAC, 16/19: rIFG, 20/25: rDLPFC, 26/30: rSAC.

7. Organization

7.1. Publication guidelines

The dissertation includes two published studies and one study accepted for publication in May 2022. According to the publication guidelines of Spie (Neurophotonics) (https://www.spiedigitallibrary.org) and Springer Nature (Scientific Reports) (https://www.nature.com), the original published texts, figures, and tables may be used by the author in the context of a dissertation.

Publication 1:
Comparison of speed versus complexity effects on the hemodynamic response of the Trail Making Test in block designs

David Rosenbaum, Leonore Blum, Paul Schweizer, Andreas J. Fallgatter, Martin J. Herrmann, Ann-Christine Ehlis, Florian G. Metzger, " Comparison of speed versus complexity effects on the hemodynamic response of the trail making test in block designs," *Neurophoton* 5 (4), 045007 (2018), doi: 10.1117/1. NPh.5.4.045007.

Publication 2:
Age-related deterioration of performance and increase of cortex activity comparing time- versus item-controlled fNIRS measurement

Leonore Blum*, David Rosenbaum, Benjamin Röben, Katja Dehnen, Walter Maetzler, Ulrike Suenkel, Andreas J. Fallgatter, Ann-Christine Ehlis, Florian G. Metzger, "Age-related deterioration of performance and increase of cortex activity comparing time- versus item-controlled fNIRS measurement," *Sci Rep* 11**,** 6766 (2021). https://doi.org/10.1038/s41598-021-85762-w

Publication 3:

Effects of ageing on functional connectivity in a neurodegenerative risk cohort: Resting state versus task measurement using functional near-infrared spectroscopy

Leonore Blum*, Anna Hofmann*, David Rosenbaum, Morad Elshehabi, Ulrike Sünkel, Andreas J. Fallgatter, Ann-Christine Ehlis, Florian G. Metzger, "Effects of ageing on functional connectivity in a neurodegenerative risk cohort: Resting state versus task measurement using functional near-infrared spectroscopy," *Sci Rep*, (2022).

7.2. Formatting

The dissertation was formatted according to the specifications of the "Merkblatt für Doktoranden und Betreuer" of the Medizinische Fakultät Tübingen (September 2017)". The citation method used (APA-6th) includes the entire dissertation. Articles already published with a different citation style have been transformed accordingly and may therefore differ from the published version. The numbering of the tables and figures has also been adapted according to the consecutive numbering of the dissertation.

7.3. Eidesstattliche Erklärung

Ich, Leonore Camilla Blum, erkläre hiermit, die vorgelegte Dissertation mit dem Titel „**Cerebral ageing: Neurophysiological correlates of ageing processes in cortical activation and functional connectivity"** verfasst zu haben und nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet zu haben.

PD Dr. Florian Metzger und Prof. Andreas J. Fallgatter überließen mir das Thema und betreuten die Arbeit. Außerdem half mir Dr. David Rosenbaum bei der Konzeption und begleitete die Arbeit.

Die Daten der TREND-Studie, die in Studie 2 und Studie 3 verwendet wurden, habe ich vom TREND-Study Consortium der Abteilung Psychophysiologie und optische Bildgebung der Klinik für Psychiatrie und Psychotherapie Tübingen zur Verfügung gestellt bekommen. Die Datenerhebung für Studie 1 erfolgte in Zusammenarbeit mit Dr. David Rosenbaum.

Ich erkläre hiermit, dass ich die Dissertationsschrift eigenständig erarbeitet und angefertigt habe. Die Inhalte, Ergebnisse und Bilder der Publikationen von Studie 2 und Studie 3 wurden selbstständig erarbeitet und verfasst. Bei der ersten veröffentlichten Studie wirkte ich als Ko-Autor bei der Verfassung, sowie bei der Datenerhebung mit. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Die Arbeit habe ich selbständig und ohne unzulässige fremde Hilfe angefertigt. Die verwendeten Quellen und Hilfsmittel sind im Literaturverzeichnis vollständig aufgeführt. Ich versichere alles kenntlich gemacht zu haben, was aus Arbeiten anderer unverändert oder mit Abänderungen übernommen wurde.

Schaffhausen, den 12. Juni 2023