Multimodal neuroimaging of prefrontal cortex (dys)function: EEG, fNIRS, fNIRS-fMRI and Imaging Genetics approaches

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I hereby declare that my thesis entitled "**Multimodal neuroimaging of prefrontal cortex (dys)function: EEG, fNIRS, fNIRS-fMRI and Imaging Genetics approaches**" is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I verify that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Tübingen .....

The publications included in the present cumulative dissertation are:

Study #1:

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Study #2

Heinzel, S., Metzger, F.G., Ehlis, A.C., Korell, R., Alboji, A., Haeussinger, F.B., Hagen, K., Maetzler, W., Eschweiler, G.W., Berg, D., Fallgatter, A.J., 2012.

Aging-related cortical reorganization of verbal fluency processing: a functional near-infrared spectroscopy study. *Neurobiology of Aging*, in press.

Study #3

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Variability of (functional) hemodynamics as measured with simultaneous fNIRS and fMRI. submitted to: NeuroImage (date: 31.08.2012, manuscript number: NIMG-12-2025).

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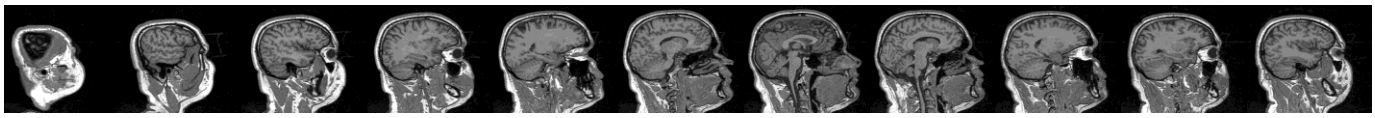
Content



Summary

The present cumulative dissertation comprises three neuroimaging studies using different techniques, functional tasks and experimental variables of diverse nature to investigate human prefrontal cortex (PFC) (dys)function as well as methodological aspects of functional near-infrared spectroscopy (fNIRS).

(1) Both dopamine (DA) availability ("inverted U-model") and excitatory versus inhibitory DA receptor stimulation ("dual-state theory") have been linked to PFC processing and cognitive control function. Electroencephalography (EEG) was recorded during a Go/NoGo response inhibition task in 114 healthy controls and 181 adult patients with attention-deficit/hyperactivity disorder (ADHD). As a neural measure of prefrontal cognitive response control the anteriorization of the P300 centroid in No-Go- relative to Go-trials (NoGo anteriorization, NGA) was investigated for the impact of genetic polymorphisms modulating catechol-O-methyltransferase efficiency (*COMT,* Val158Met) in degrading prefrontal DA and inhibitory DA receptor D4 sensitivity (*DRD4*, 48bp VNTR). Single genes and ADHD diagnosis showed no significant impact on the NGA or behavioral measures. However, a significant *COMT×DRD4* interaction was revealed as subjects with relatively increased D4-receptor function (*DRD4*: no 7R-alleles) displayed an "inverted U"-relationship between the NGA and increasing *COMT*-dependent DA levels, whereas subjects with decreased D4-sensitivity (7R) showed a U-relationship. This interaction was supported by 7R-allele dose-effects and also reflected by an impact on task behavior, i.e. intraindividual reaction time variability. Combining previous theories of PFC DA function, neural stability at intermediate DA levels may be accompanied by the risk of overly decreased neural flexibility if inhibitory DA receptor function is additionally decreased. The findings of *COMT×DRD4* epistasis might help to disentangle the genetic basis of dopaminergic mechanisms underlying prefrontal (dys)function.

(2) While progressive neurocognitive impairments are associated with aging and Alzheimer's disease (AD), cortical reorganization might delay difficulties in effortful word retrieval, which is one of the earliest cognitive signs of AD. Therefore, cortical hemodynamic responses were measured with fNIRS during phonological and semantic verbal fluency, and investigated in 325 non-demented, healthy subjects (age: 51-82

years). The predictive value of age, sex, verbal fluency performance and years of education for the cortical hemodynamics was assessed using multiple regression analyses. Age predicted bilaterally reduced inferior frontal junction (IFJ) and increased middle frontal and supramarginal gyri activity in both task conditions. Years of education as well as sex (IFJ activation in females > males) partly predicted opposite effects on activation compared to age, while task performance was not a significant predictor. All predictors showed small effect sizes ($-.24 < \beta < .22$). Middle frontal and supramarginal gyri activity may compensate for an aging-related decrease in IFJ recruitment during verbal fluency. The findings of aging-related (compensatory) cortical reorganization of verbal fluency processing might, in combination with other (risk) factors and using longitudinal observations, help to identify neurodegenerative processes of Alzheimer's disease, while individuals are still cognitively healthy.

(3) Individual anatomical or systemic physiological sources of variance may hamper the interpretation of fNIRS signals as neural correlates of cortical functions and their association with individual personality traits. Using simultaneous fNIRS and functional magnetic resonance imaging (fMRI) of hemodynamic responses elicited by an intertemporal choice task in 20 healthy subjects, variability in crossmodal correlations and divergence in associations of the activation with trait "sensitivity to reward" (SR) was investigated. Moreover, an impact of interindividual anatomy and scalp fMRI signal fluctuations on fNIRS signals and activation-trait associations was studied. Both methods consistently detected activation within right inferior/middle frontal gyrus, while fNIRS-fMRI correlations showed wide variability between subjects. Up to 41% of fNIRS channel activation variance was explained by gray matter volume (simulated to be) traversed by near-infrared light, and up to 20% by scalp-cortex distance. Extracranial fMRI and fNIRS time series showed significant temporal correlations at the temple. Trait SR was negatively correlated with fMRI but not fNIRS activation elicited by immediate rewards of choice within right inferior/middle frontal gyrus. Higher trait SR increased the correlation between extracranial fMRI signal fluctuations and fNIRS signals, suggesting that task-evoked systemic arousal-effects might be trait-dependent. Task-related fNIRS signals might be impacted by regionally and individually weighted sources of anatomical and systemic physiological error variance.

Trait-activation correlations might be affected or biased by systemic physiological arousal-effects, which should be accounted for in future fNIRS studies of interindividual differences.

Zusammenfassung

Die vorliegende kumulative Dissertation umfasst drei funktionelle Bildgebungsstudien, welche mit unterschiedlichen methodischen Verfahren, Versuchsaufgaben und experimentellen Variablen Hirnfunktionen des präfrontalen Kortex sowie methodische Aspekte der funktionellen Nahinfrarotspektroskopie (fNIRS) untersuchten.

(1) Sowohl die präfrontale Dopamin (DA)-Verfügbarkeit ("inverted U-model") als auch das Verhältnis der Stimulation von exzitatorischen und inhibitorischen DA-Rezeptoren ("dual-state theory") wurde mit präfrontaler Verarbeitung und Funktionen wie kognitiver Kontrolle in Verbindung gebracht. Während der Bearbeitung einer Aufgabe zur motorischen Antworthemmung wurden die elektrischen Hirnsignale mittels Elektroenzephalographie (EEG) bei 114 gesunden Probanden und 181 adulten Patienten mit Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung abgeleitet. Als neuronales Maß der präfrontalen kognitiven Antwortkontrolle wurde die Anteriorisierung der P300-Zentroide während NoGo- relativ zu Go-Aufgabenbedingungen verwendet (NoGo-Anteriorisierung, NGA). Die NGA wurde hinsichtlich eines Einflusses von genetischen Polymorphismen untersucht, welche den DA Abbau durch die Katechol-O-Methyltransferase (*COMT*, Val158Met) bzw. die DA D4-Rezeptorsensitivität (*DRD4*, 48 bp VNTR) modulieren. Während die NGA weder Gen-Haupteffekte noch Unterschiede zwischen Gesunden und Patienten zeigte, war eine signifikante epistatische *COMT×DRD4* Interaktion zu beobachten. Personen mit relativ gesteigerter D4-Rezeptorsensitivität (kein 7R-Allel) zeigten einen umgekehrten U-Zusammenhang zwischen der NGA und steigender *COMT*-abhängiger DA-Verfügbarkeit, wohingegen Personen mit relativ verringerter D4-Rezeptorsensitivität (7R-Allel) einen U-Zusammenhang zeigten. Diese Gen-Gen Interaktion zeigte *DRD4* 7R-Alleldosis-Effekte und spiegelte sich auch behavioral in der intraindividuellen Go-Reaktionszeitvariabilität wider. Neuronale Stabilität bei mittlerer DA-Verfügbarkeit

könnte mit einem erhöhen Risiko verringerter Flexibilität einhergehen, wenn zusätzlich die inhibitorische DA D4-Rezeptorfunktion eingeschränkt ist. Über die gezeigte Interaktion genetischer Einflussvariablen vereinigen die Ergebnisse bestehende Theorien zur DA-Verfügbarkeit bzw. dem Verhältnis DA-abhängiger neuronaler Erregung und Hemmung mit Einfluss auf präfrontale kognitive Kontrolle.

(2) Alterungsprozesse und die Alzheimer-Demenz sind mit Beeinträchtigungen neurokognitiver Funktionen verbunden, wobei eine verringerte Wortflüssigkeit zu den frühesten Symptomen der Alzheimer-Demenz gehört. Kompensatorische Prozesse, welche diesen Symptomen (zunächst) entgegenwirken, können sich in einer Reorganisation kortikaler Verarbeitung zeigen. Zur Untersuchung dieser Prozesse wurden kortikale hämodynamische Antworten während der phonologischen und semantischen Wortflüssigkeit wurden bei 325 nicht-dementen gesunden Personen (Alter: 51-82 Jahre) mittels fNIRS untersucht. Der prädiktive Wert von Alter, Geschlecht, Wortflüssigkeitsleistung und der Ausbildungsjahre der Versuchspersonen bezüglicher der kortikalen hämodynamischen Antworten wurde mittels multipler Regression untersucht. Das Alter war ein signifikanter Prädiktor reduzierter bilateraler Aktivität im Übergangsbereich vom inferior frontalen Gyrus zum temporalen Pol (IFT) und gesteigerter bilateraler Aktivität im mittleren frontalen und supramarginalen Gyrus. Die Ausbildungsjahre und das Geschlecht (IFT-Aktivität bei Frauen höher als bei Männern) zeigten teilweise dem Alter entgegengesetzte Effekte, während die Wortflüssigkeitsleistung keinen signifikanten Einfluss hatte. Alle Prädiktoren zeigten nur kleine Effektstärken ($-.24 < \beta < .22$). Die gesteigerte Aktivität im mittleren frontalen und supramarginalen Gyrus könnte einen Kompensationsprozess für gesenkte IFT Aktivität mit steigendem Altern darstellen. Diese Belege einer (kompensatorischen) kortikalen Reorganisation der Verarbeitung von Wortflüssigkeit könnten, in Kombination mit weiteren (Risiko-)Faktoren und im Rahmen longitudinaler Untersuchungen, dazu beitragen neurodegenerative Prozesse einer Alzheimer-Demenz zu erkennen, bevor erste kognitive Symptome erkennbar sind.

(3) Einflüsse individueller Anatomie und systemischer physiologischer Artefakte können die Validität der Interpretation von fNIRS Signalen als Korrelate kortikaler Hirnaktivität und Korrelationen dieser Aktivität mit individuellen (Persönlichkeits-)Maßen

einschränken. Zur Untersuchung dieser Problematik wurde eine simultane Messung hämodynamischer Antworten mit fNIRS und funktioneller Magnetresonanztomographie (fMRT) bei 20 gesunden Versuchspersonen durchgeführt, während eine Entscheidungsaufgabe zwischen Geldbeträgen unterschiedlicher Höhe und Aushändigungszeitpunkte durchgeführt wurde. Beide Methoden zeigten konsistente Aktivierung im rechten inferioren/mittleren frontalen Gyrus. Korrelationen der fNIRS mit den fMRT Zeitreihen zeigten jedoch eine hohe Variabilität zwischen den Versuchspersonen. Bis zu 41% der Varianz der fNIRS-Aktivität wurde durch das simulierte individuelle Volumen der von fNIRS erfassten grauen Hirnsubstanz eines Messkanals, und bis zu 20% durch den Abstand zwischen Kopfoberfläche und Kortex, aufgeklärt. Die fMRT-Zeitreihen in der Haut zeigten zudem signifikante Korrelationen mit dem fNIRS-Signal in der Schläfenregion. Während fMRT eine signifikante negative Korrelation der inferioren/mittleren frontalen Gyrus-Aktivität mit dem Persönlichkeitsmerkmal "Belohnungssensitivität" zeigte, war die Korrelation bei fNIRS nicht signifikant. Eine erhöhte Belohnungssensitivität erhöhte zudem die Korrelation zwischen fNIRS und fMRT in der Haut, welches auf eine durch Erregung erhöhte systemisch-physiologische Reaktion in Abhängigkeit des Persönlichkeitsmerkales hindeuten könnte. Die mit fNIRS aufgezeichneten hämodynamischen Antworten unterliegen regionaler und individuell-gewichteter anatomischer und systemisch-physiologischer Fehlervarianz und zukünftige fNIRS-Studien zu interindividuellen Unterschieden sollten diesen Umstand berücksichtigen.

Abbreviations

ACC	**Anterior cingulate cortex**
AD	**Alzheimer's disease**
BOLD	**Blood-oxygenation-level-dependent**
CNS	**Central nervous system**
COMT	**Catechol-O-methyltransferase**
CSF	**Cerebrospinal fluid**
DA	**Dopamine**
Deoxy	**Deoxygenated hemoglobin**
DLPFC	**Dorsolateral prefrontal cortex**
DNA	**Deoxyribonucleic acid**
DSM	**Diagnostic and Statistical Manual of Mental Disorders**
DTI	**Diffusion tensor imaging**
EEG	**Electroencephalography**
EPI	**Echo-planar imaging**
ERP	**Event-related potential**
FDR	**False discovery rate**
fMRI	**Functional magnetic resonance imaging**
fNIRS	**Functional near-infrared spectroscopy**
FWE	**Family-wise error**
GABA	**γ-Aminobutyric acid**
HHb	**Deoxygenated hemoglobin**
IFJ	**Inferior frontal junction**
ITC	**Intertemporal choice**
MCI	**Mild cognitive impairment**

MRI	**Magnetic resonance imaging**
NGA	**NoGo-anteriorization**
O_2Hb	**Oxygenated hemoglobin**
OFC	**Orbitofrontal cortex**
Oxy	**Oxygenated hemoglobin**
PFC	**Prefrontal cortex**
ROI	**Region of interest**
RT	**Reaction time**
SCD	**Scalp-cortex distance**
SCID	**Structured interview for DSM-IV**
SD	**Standard deviation**
SEM	**Standard error of the mean**
SNP	**Single nucleotide polymorphism**
SR	**Sensitivity to reward**
VBM	**Voxel-based morphometry**
VFT	**Verbal fluency test**
V_{gray}	**Volume of gray matter absorbing light**
VMPFC	**Ventromedial prefrontal cortex**
VNTR	**Variable-number of tandem repeat**

General introduction

The peripheral and central nervous system (CNS) serve a plethora of functions, which ultimately regulate the body system and its interaction with the environment. Information processing mediated by neural networks are fundamental to these functions – from basic cellular and physiological homeostasis of various organ systems, sensory processes, cognitive functions underlying complex decision-making processes, to motor processes underlying behavior. Thereby, these neural systems allow animals to adapt and respond as relevant intrinsic or external parameters of the functioning of body or mind change over time.

"… to move things is all that mankind can do, for such the sole executant is muscle, whether in whispering a syllable or in felling a forest." (Charles Sherrington, 1924)

In higher vertebrate and especially humans, the prefrontal cortex (PFC) plays a central role for the neural processes of cognitive control and (executive) functions underlying behavior (Fuster, 2008a; Miller and Cohen, 2001). Cognition and behavior are vastly defined by the neural networks of the PFC and its connectivity with other brain regions, which have been sculpted by environmental and (epi)genetic influences.

The present cumulative dissertation comprises studies of various neuroimaging techniques investigating cognitive control and executive functions via task-related activation of the PFC and its modulation by (1) molecular genetic factors and psychopathology, (2) demographic and behavioral factors, and (3) personality traits. Moreover, optical neuroimaging of PFC functional hemodynamics is examined in regard to potential confounding factors of systemic physiological hemodynamic influences and an impact of individual anatomy. These different factors investigated in the light of PFC activation and function, as well as neuroimaging methodology, are of highly diverse nature in respect to their role within the principal organization of the human CNS. To give a general overview of the conceptually discrete, yet functionally highly interconnected entities – from genes to personality – the principal organizational

levels of the human CNS are briefly introduced: From the genetic and molecular level to cells to cellular networks to brain functions, behavior and personality.
Thereafter, an introduction to the PFC and its functions as well as neuroimaging methods and approaches involved in the studies of the present cumulative dissertation is given.

Organizational levels of the CNS

While the genetic information in form of deoxyribonucleic acid (DNA) principally codes for protein elements of all cells, many possible mechanisms may regulate the expression of a gene coding for a particular protein. The emerging patterns of gene expression are specific to cell types, and thereby largely define the cellular phenotypic identity. Gene expression is modulated at the level of transcription, mRNA processing and translation, however, involving a multitude of (epi)genetic mechanisms such as the regulation of DNA-histone complexation and DNA methylation, the binding of transcription factors and other proteins to the DNA and various post-transcriptional regulations (Graw, 2006). Therefore, (epi)genetic mechanisms play a key role in determining cellular function. The (epi)genetic factors can, for instance, impact on neuronal or glial function by modulating differentiation, growth and morphogenesis, "housekeeping" function, (synaptic) plasticity, and neurotransmission (Bilder et al., 2004; Fagiolini et al., 2009; Homberg and Lesch, 2011; Olynik and Rastegar, 2012). Also, genetic variability mediated by single nucleotide polymorphisms (SNPs), variable-number-of-tandem-repeat (VNTR) polymorphisms, gene copy number variation (CNV), microsatellites, and deletions/insertions of DNA regions may either change transcriptional activity or translational efficiency, or protein conformation and function. Thereby, genetic variants can modulate various functional processes of cellular function, such as neurotransmission. In this regard, a functional role of genetic variants has been implicated for, e.g. neurotransmitter synthesis (*TPH2*), vesicular release (*SNAP25*), synaptic reuptake (*5-HTT*, *DAT*) or catabolism (*COMT, MAOA*), as well as post- and presynaptic receptor function (*DRD4*, *DRD2*),

ion channel function (*KCNJ6*) or signal transduction cascades (*DARPP32*). Moreover, genetic variation of genes for neurotrophic factors (*BDNF*), plasticity/transcription factors (*CREB1*), neuropeptides (*NPY*, *NPS*), apolipoproteins (*APOE*) and many others likely impact important processes of cellular or CNS function (Allen et al., 2008; Arcos-Burgos et al., 2012; Gizer et al., 2009; Levinson, 2006; Miyakawa, 2007).

The organizational level of molecular (epi)genetics (in interaction with the extracellular environment) defines the molecular, morphological and functional identity of cells. The cellular phenotypic details allow for a differentiation of up to 411 distinct cell types in the adult human body, with at least 145 types of neurons in the adult human brain (Vickaryous and Hall, 2006). However, each cell is unique and constantly changing. Thus, the individuality of each human brain and each individual personality may only in part be emergent from the mere number of CNS cells and the vast number of possible structural connections and functional interactions. The adult human brain has recently been estimated to contain 86 billion neurons, with 16.3 billion in the cerebral cortex, and 69 billion in the cerebellum (Herculano-Houzel, 2009). Overall, glia cells represent approximately half of all brain cells, however, large interregional differences in regard to the glia-to-neuron ratio have been reported (e.g., cerebral cortex gray matter: 2:1, cerebellum: 1:25, thalamus: 17:1) (Andersen et al., 1992; Azevedo et al., 2009; Pelvig et al., 2008). Estimates of an average of 7000 chemical synapses connecting onto a neocortical neuron have been reported for the (young) adult brain (Pakkenberg et al., 2003). Neurons can transfer information to other cells (or to themselves via autoreceptors or recurrent axons) using a multitude of different chemical agents, including neurotransmitters, neuropeptides, cannabinoids, and gaseous transmitters. About 100 different peptides are known to be released by different populations of neurons in the mammalian brain (See: Neuropeptide Database, an internet resource summarising all known neuropeptides, their genes, precursors and expression in the brain: http://www.neuropeptides.nl). Major neurotransmitters are acetylcholine, glutamate and γ-aminobutyric acid (GABA), and furthermore, dopamine, noradrenaline and serotonin, which often have a neuromodulatory rather than a direct neurotransmission function. In regard to their (postsynaptic)

effect neurotransmitters can be categorized as inhibitory or excitatory with glutamate being the most abundant excitatory and GABA being the major inhibitory neurotransmitter in the (adult) vertebrate CNS (Kandel et al., 2000a). However, depending on postsynaptic receptors, the same neurotransmitter may have excitatory, inhibitory as well as modulatory functions in (synaptic) neurotransmission. Specifically, the local effect of released chemical agents is defined and mediated by the transient binding to specific ionotropic or metabotropic receptor types. Most commonly, synaptic boutons release transmitters onto dendritic spines where the binding to (1) ionotropic receptors triggers the opening of ion channels and the subsequent flow of ions alters the electrochemical membrane potential. Thereby, temporal and spatial information is integrated into the dendrite. The change in membrane polarization may, in concert with other inputs, sufficiently depolarize the soma's axon hillock to generate an action potential propagating along the axon, which then itself might trigger the synaptic release of (neuro)transmitters onto postsynaptic partners. (2) Metabotropic receptors trigger intracellular (second-messenger) signaling cascades modulating, for instance, ion-channel activation/inhibition or gene transcription (Kandel et al., 2000b). The potential effects of the multitude of chemical signaling agents are multiplied by the diversity of receptor- and ion channel-isoforms (including alternative splicing variants) with specific binding, conformational and kinetic characteristics. Gene expression of different receptor types may not only be specific to particular brain regions, but stages of neural development are accompanied with the expression of, for instance, different glutamate and GABA-receptor isoforms and receptor subunit compositions (Lujan et al., 2005).

Glial cells such as astrocytes play an important role in physical support, oxygen and glucose supply via neurovascular coupling and myelin insulation of neurons. Also, accumulating evidence suggests a central role for astrocytes in the control of neuronal synaptic transmission (Haydon and Carmignoto, 2006), adding another dimension to the complexity of neural processing. Recently, astrocytes have been shown to release neurotransmitters and peptides in vicinity to neuronal synapses, e.g. in response to declining extracellular calcium levels, thereby reciprocally communicating and modulating neuron-astrocyte transmission (Araque and Navarrete; Torres et al.,

2012). Moreover, neurons as well as astrocytes often communicate through gap junctions (electrical synapses) connecting the cytoplasm of neighboring cells. This allows for an exchange of (calcium) ions or small molecules such as adenosine triphosphate (ATP). For hippocampal and neocortical neurons gap junctions, amongst other functions, are involved in the synchronization of rhythmic oscillations of activity important for memory formation and consolidation (Dere and Zlomuzica, 2012).

Sensory perception, motor behavior, every thought and mental representation and, ultimately, the individual personality of a human being is the result of information processing mediated by interconnected neuronal and glial cell assemblies and networks within the CNS and the peripheral nervous system.
150,000 to 180,000 km cumulative length of myelinated nerve fibers have been reported for the (young) adult brain (about 80,000 km at an age of 80 years) enabling extensive interneuronal information transfer (Marner et al., 2003).
The comprehensive structural description of these networks of elements and connections in the human brain is referred to as the human connectome (Sporns et al., 2005). The structural connectivity of the brain encompasses multiple scales of organization. From the microscale of cells and synapses to small neural networks to macroscopic nerve fiber bundles and morphological characteristics of interconnected brain regions (Sporns, 2011).
Despite the vast complexity of neural networks some general structural characteristics of the brain are apparent. First, for the mammalian cerebral cortex six distinctive layers can be defined based on cytoarchitecture. Each layer is composed of dendritic, somatic or axonal compartments of different neuronal cell types, such as glutamatergic projection neurons and GABAergic local interneurons. Importantly, the laminar structures differ in connectivity of projection neurons to e.g., subcortical, intracortical or thalamic regions (Kandel et al., 2000c). Regions of the cerebral cortex do not differ in the general layer composition but in the prominence of particular layers. Second, a vast literature describes a modular neocortical organization in the connection of neurons in forms of columns of about 80 to 100 neurons. However, concepts and definitions of these minicolumns as well as of macro-, and hyper-columns vary

between species, brain regions, and response properties, shared input, and common output. Therefore, the fundamentals of the function, the structural varieties and common processing mechanisms of columnar organization are far from being understood (DeFelipe et al., 2012; Horton and Adams, 2005). Recently, synaptic clustering in rats has been shown to organize a few dozen neocortical (pyramidal) neurons into mini-circuits (Perin et al., 2011). Within these 'elementary' neural building blocks the connectivity between cells had less than two degrees of separation while the number of connections was directly proportional to the number of common neighbor cells. Importantly, the connections were innate in all investigated animals rather than formed by experience, suggesting that these mini-circuits might be combined into high order constructs underlying acquired experience or memory.

On a more macroscopic level of interregional nerve fiber connections, for instance, computational tractography and diffusion tensor imaging (DTI) using non-invasive magnetic resonance imaging (MRI) have been used to generate atlases of the human connectome (Mori et al., 2009). Briefly, a set of six major network modules has been identified within the frontal, temporoparietal and medial cortex (Hagmann et al., 2008). These modules are coupled through highly connected hub-nodes largely positioned along the anterior-posterior medial axis including rostral and caudal anterior cingulate cortex, paracentral lobule and precuneus (Gong et al., 2009; Iturria-Medina et al., 2008). A similar network and hub distribution has been found for the cat and macaque monkey CNS (Zamora-Lopez et al., 2010).

While many consistencies between structural and functional connectivity of network modules and hubs have been shown (Greicius et al., 2009), it is important to note that the functional connectivity between two brain regions reflects a combination of direct and indirect network paths. Thus, structural connectivity does not rigidly determine neural dynamic interactions, but reduces the (spatial) dimensionality in which neural states can be represented, thereby allowing for fluid and variable neural processing which is sensitive to neural perturbations (Sporns, 2011). Moreover, the structural and functional neural levels are reciprocally linked through a variety of mechanisms of plasticity (Rubinov et al., 2009). Importantly, in addition to the association of clinical conditions such as Alzheimer's disease or schizophrenia to alterations

in hub and network structures, the connectome shows large interindividual variability. Currently, several major projects investigate the micro- and macroscopic levels of the connectome in humans and other mammals, its genetic heritability, its relation to neural processing mechanisms, dynamic patterns of resting state and task-related brain activation, characteristic alterations in neurological and psychiatric conditions and the interindividuality of connectomes, to ultimately connect the organizational levels of the brain to behavior, cognition and personality.
For recent developments in major human neuroscience and brain mapping projects see, e.g.: www.humanconnectomeproject.org

www.brain-map.org

www.humanbrainproject.eu

The psychological study of mental functions and behavior can be inherently linked to both, the science of their (developing) biological substrates as well as to, for instance, sociology, philosophy, cultural sciences, individual and cultural history, and many facets of environmental and evolutionary influences. To quantitatively relate individual cognitive processes, (personality) traits or behavior to physiological processes, various experimental psychological methods can be used. For instance, psychometric assessments can involve self-report inventories based on personality theories, clinical symptoms or behavior. Another approach is the operationalization of behavior using standardized empirical observations. Depending on the task design and the dependent and independent variables these experiments may investigate and quantify behavioral and cognitive processes. Many of these behavioral and cognitive experiments can be adapted to experimental designs suited for the simultaneous measurement of various physiological responses which precede, underlie or follow these behavioral or cognitive processes. Beside various functional neuroimaging methods assessing (surrogate) measures of neural processes in the brain, many peripheral physiological parameters can be assessed such as heart rate, blood pressure, (facial) muscle or eye movements, skin temperature and conductance, or hormone levels to name but a few.

The degrees of freedom which may underlie individual variability are countless as they emerge from the bottom of each of the organizational entities of the brain as well as through their interaction. Despite this complexity, the engagement of specific brain regions and their interaction with other regions has been identified to underlie functional roles for behavior and cognition. Herein, the PFC plays a pivotal role.

The prefrontal cortex (PFC)

The PFC comprises several widely interconnected neocortical areas with reciprocal projections from virtually all sensory systems, motor systems including (sub)thalamic and cerebellar connections, and many subcortical regions such as limbic and mid-brain structures involved in reward, memory and emotional processing (Fuster, 2008b). The extensive reciprocal connectivity with other neural systems enable, for instance, multimodal convergence of visual, somatosensory, and auditory information, and direct or indirect integration of this information into the PFC, and the top-down modulatory control of the PFC over other regions, which is key to its diverse functions (Miller and Cohen, 2001; Miller et al., 2002).
While distinct PFC areas can be differentiated by morphological or cytoarchitectonic characteristics, PFC compartmentalizations may not strictly indicate their functional specialization, which rather emerges from the cooperative interaction, i.e. the structural and functional connectivity, of a particular region with other neural structures (Fuster, 2008d). Beside specific roles which have been implicated for some distinct PFC compartments (e.g., Broca area, (pre)motor area, frontal eye fields), a rough functional distinction between lateral, medial and ventral (orbitofrontal) PFC is well supported by differences in connectivity patterns and a wealth of lesion, cellular recording, neuroimaging and neuropsychological studies in primates and humans (Fuster, 2008c).

The lateral PFC plays an important role in executive behavioral control, which is enabled by (1) the collection and integration of information by its direct connections

to association cortex, limbic cortex, and subcortical structures or indirect connections through orbital and medial PFC structures, (2) the modulation and adaptive control of information flow through cortical and subcortical structures, and (3) the connections of the lateral PFC to premotor areas, the basal ganglia, and the cerebellum which support key aspects of motor behavior. Thereby, the lateral PFC encompasses a multitude of functions as for instance, attentional control, working memory, decision-making and integrative goal-directed action planning and selection, response inhibition, and emotion- and reinforcer-based behavioral control (Tanji and Hoshi, 2008). Dysfunction of the lateral PFC or its functional/structural connectivity to other regions has been implicated in psychopathological symptoms (and comorbidities of pathologies), such as self-regulation failure of appetitive behaviors in addiction, emotion regulation in depression, or deficits in various aspects of cognitive/executive functions in Alzheimer's disease, schizophrenia or ADHD (Heatherton and Wagner, 2011; Millan et al., 2012).

The orbitofrontal cortex (OFC) and parts of the ventromedial PFC (VMPFC) have dense connections with all sensory areas, limbic structures such as extensive reciprocal connections to the amygdala, insula and hippocampus and (ventromedial) striatum, moreover to the thalamus, hypothalamus, brainstem and dorsolateral PFC (Barbas, 2007; Cavada et al., 2000). Corresponding to these structural connections the OFC/VMPFC represents an important nexus for sensory integration, emotional processing, and hedonic experience (Kringelbach and Rolls, 2004), but also for reward- and punishment-guided learning, (subjective) evaluation, decision-making and maintenance of successful choices, as well as regulation of autonomic functions (Berridge and Kringelbach, 2008; Grabenhorst and Rolls, 2011; Noonan et al., 2012; Schoenbaum et al., 2011). While the OFC/VMPFC is a highly complex structure in regard to its functional interactions with other regions, its dysfunction has been linked to, for instance, impulsivity/compulsivity, addiction, obsessive–compulsive disorder (Robbins et al., 2012), and affective dysregulation, such as bipolar disorder and major depression (Cotter et al., 2005).

The (dorso)medial PFC including the anterior cingulate cortex (ACC) is integrated in both cognitive-behavioral neural networks and emotional-autonomic-motor networks

(Bush et al., 2000), and is thereby involved in emotional as well as cognitive processing. For instance, the ACC is crucial for the monitoring for processing and response conflicts and errors, and for mediating the recruitment of control functions of the DLPFC which may implement appropriate behavioral adjustments (Ridderinkhof et al., 2004a; Ridderinkhof et al., 2004b). Moreover, the (dorso)medial PFC and the ACC are important for the processing of emotional conflict regulation, social cognition and pain (Amodio and Frith, 2006; Etkin et al., 2011). For bipolar disorder and schizophrenia the medial PFC/ACC has been shown to be functionally decoupled from the DLPFC, while in bipolar disorder additionally a stronger coupling of this region and the insula and ventrolateral PFC was shown, indicating the cognitive and emotional deficits symptomatic for these disorders (Chai et al., 2011).

The studies included in the cumulative dissertation largely focused on the investigation of prefrontal cognitive control and executive functions involving the lateral PFC and the ACC. Neural correlates of these prefrontal functions were investigated in different task situations during the functional neuroimaging measurements: (1) Motor response inhibition requiring quick button response to "Go" stimuli and inhibition of that response to "NoGo" stimuli, (2) verbal fluency involving the effortful retrieval of lexical representations corresponding to phonological or semantic criteria, and (3) intertemporal choice between monetary reward options which differed in the amount and delay-to-delivery, i.e. smaller/sooner versus larger/later rewards.

Techniques for the investigation of neural functions

Over the last decades, major strides in neuroscience and its many subdisciplines were often preceded by methodological advancements regarding measurement and computation techniques as well as analytical methods and experimental designs. Various methods allow for the investigation of neural functions at different levels. By using invasive voltage/patch-clamp or intra- and extracellular electrophysiological recordings, calcium imaging or optogenetic approaches, the activity of neurons can

be directly investigated (Scanziani and Hausser, 2009). While these invasive techniques are mostly used in animal research, they allow to temporally and spatially precisely study the behavior of neuronal activity in the micro- to millisecond range. Non-invasive recordings of electrical neural activity suited for human research, such as electroencephalography (EEG) or magnetoencephalography (MEG), however, record the combined electrical activity of large neuronal assemblies comprising many thousands of neurons (Bagic and Sato, 2007). Other non-invasive modalities use surrogate measures of neural activity such as the blood oxygenation level dependent (BOLD) signal. Through astrocyte-mediated neurovascular coupling of neural activity with a local vascular response increasing blood flow, volume and oxygenation, the neural activity can be indirectly monitored (Logothetis, 2002). This process limits the temporal resolution to several seconds thereby putting a cap on the temporal resolution of methods such as functional magnetic resonance imaging (fMRI) (Bandettini, 2007) or functional near-infrared spectroscopy (fNIRS) (Ferrari and Quaresima, 2012; Hoshi, 2003; Plichta et al., 2007; Steinbrink et al., 2006). The spatial resolution in the millimeter range for fMRI and centimeter range for fNIRS only allows inferring neural function of relatively large neural assemblies comprising several million neurons. Using voxel-based morphometry (VBM) or DTI in MRI the technique also allows for structural and connectivity analyses of the brain. Moreover, positron emission tomography (PET) or single-photon emission computed tomography (SPECT) measure the isotope decay of radioactively labeled molecules such as glucose or H_2O, which can be detected in the brain after injection into the bloodstream. Thereby, metabolism or blood flow indicative of a local increase in neural activity can be studied with a spatial resolution of millimeters and a temporal resolution of several minutes (Gulyás and Sjöholm, 2007). Considering the experimental design of a functional task, stimulation or resting state performed during the functional neuroimaging recordings may allow to indirectly investigate specific brain functions, neural processing and engagement of brain regions during the experiment.

The neuroimaging techniques used in the studies of this cumulative dissertation – EEG, fNIRS and fMRI as well as Imaging Genetics – are briefly introduced.

Electroencephalography (EEG)

EEG can record the collective electrical activity resulting from extracellular ionic current flows mediated by neuronal assemblies comprising tens of thousands of (pyramidal) neurons. Voltage changes (~ 10-100 µV) recorded between EEG electrodes on the scalp surface mostly reflect action potentials propagating along myelinated axons oriented radially relative to the scalp. The electrical potential between an electrode recording neural activity and a reference electrode in an inactive area (unipolar recording), or between two electrodes recording neural activity (bipolar recording), can be measured. However, localization of the electrical source faces the "inverse problem" in EEG, i.e. the extracranially recorded potentials can have many different possible sources within the brain and the spatial location of the source of the EEG signal can only be (ambiguously) estimated following multiple assumptions about the impact of these possible generators on the recorded signal ("forward problem"). Continuous recordings of the multiple EEG channels can be analyzed with respect to their wave patterns and frequency power spectra to infer neural states from neural oscillations and spike rates in resting state or during the performance of a particular task (Bagic and Sato, 2007). Specific task-related neural functions are often investigated using event-related potentials (ERPs). Here, the temporal sequence of task events is used to average segments of EEG signals during different experimental conditions. Thereby, signals unrelated to the event are averaged out and positive and negative voltage ERP deflections in the millisecond range following the onset of a task condition can be interpreted with respect to the task characteristics to infer neural functions. Characteristic latencies of an ERP waveform peak as well as its amplitude (1-30 µV) are often related to specific neural processes (Birbaumer and Schmidt, 2006). For instance, the P300 (positive deflection at roughly 250-500 ms after the onset of an event) has been associated with attentional processes sensitive to task processing demands and individual differences in cognitive capability. Specifically, the P300 amplitude has been associated with attentional resource allocation implicating cognitive demands during task processing (Polich, 2007).

Functional magnetic resonance imaging (fMRI)

Two major fundamental principles underlie fMRI. First, after (anti)parallel alignment of the spin vector of protons to a magnetic field the vector begins to precess in the presence of a second orthogonal magnetic field. Magnetic resonance occurs when applying radio frequency (rf) pulses with the same frequency as the proton precession, thereby increasing the nuclear spin energy (magnetization), as indicated by the flip angle of the precession vector. When switching off the rf-pulses the precession vector returns to the equilibrium state within a certain amount of time (relaxation time). The reduction of magnetization induces a current in a (receiver) rf-coil, which in combination with the relaxation times indicate physical and chemical characteristics (proton density) unique to certain tissue types of the body. To make an image, the spin's precession frequency has to be made dependent on the location of the spin, which is achieved by using different magnetic field gradients. Slice selection, and phase and frequency encoding allow for an image formation of the data recorded by the rf-coil. Ultimately, about 50.000 voxels of 1 mm^3 or smaller, depending on the maximal field strength of the MRI scanner, generate a structural image of the brain, which can be used for structural analyses such as VBM (e.g. cortical gray matter thickness, hippocampal volume, etc.) or as a template to superimpose functional images (Bandettini, 2007).

Second, fMRI is based on the differences in magnetic properties between oxygenated (O_2Hb) and deoxygenated hemoglobin (HHb). HHb is paramagnetic, whereas O_2Hb has the same magnetic susceptibility as water and brain tissue. Thus, the presence of HHb creates a local distortion of the magnetic field, and since the field strength is directly proportional to the precession frequency of protons, their precession coherence is disturbed through field inhomogenities (due to HHb) causing a relative decrease in the MRI signal. During neural activation the blood flow increases locally causing a decrease in the amount of HHb, and thus, a relative MRI signal increase of a few percent (Bandettini, 2007). This increase in the BOLD-signal can be related to the temporal sequence of a functional task condition performed during the fMRI measurements, thus indirectly indicating task-related neural activation. However,

the BOLD-response is relatively slow and is usually only detected after several seconds (~ 4-7 s) from the onset of the underlying neural activity, thereby limiting the temporal resolution of fMRI (Rosen et al., 1998).

Functional near-infrared spectroscopy (fNIRS)

fNIRS is an optical neuroimaging method that exploits (1) the transparency of biological tissues for light of wavelengths between 600-1000 nm, and (2) the characteristic absorption spectra of O_2Hb and HHb, respectively, allowing for spectroscopic differentiation. (3) Importantly, changes of the physiological state of the cerebral tissue result in negligible changes in light scattering (Obrig and Villringer, 2003; Obrig et al., 2000). Using a modified Beer Lambert law, relative concentration changes can be calculated as a function of total photon path length (Villringer and Chance, 1997). Since the length of the optical path cannot be measured by continuous wave systems, the scale unit of the change in O_2Hb and HHb, respectively, equals the concentration multiplied by the unknown path length [mmol × mm] (Hoshi, 2003). Since the path length and the tissue composition traversed by the light might differ between scalp positions, signals cannot be quantitatively compared between different fNIRS channels.

Using continuous-wave fNIRS systems, near-infrared light is constantly emitted traversing the highly scattering media of scalp, scull, cerebrospinal fluid and cortical tissue. Due to scattering and absorption, only about 0.001% of the emitted light reaches the detector which is commonly positioned on the head surface 3 cm apart from the emitter (Haeussinger et al., 2011; Okada et al., 1997). The light follows an ellipsoid path through the tissue, traversing cortical gray matter in a depth of 2-3 cm from the head surface before reaching the detector (Cui et al., 2011; Haeussinger et al., 2011). The relative changes in O_2Hb and HHb concentrations in the gray matter change the light absorption, and thereby the light intensity measured at the detector. Similar to fMRI, the BOLD-signal thereby indicates task-related neural activation of the cortex. Compared to fMRI, fNIRS has a lower spatial resolution of about 2-3 cm,

and while the fNIRS sampling rate can be sampled above 10 Hz, the relatively slow BOLD-response is a temporal limitation of measuring neural events.
To record fNIRS signals, a probe-set is positioned on the scalp which consists of, for instance, 17 light emitters and 16 detectors with 3 cm inter-optode distance creating 52 recording channels (each ~3 cm spatial resolution) covering a cortical area of interest of 6 cm × 30 cm. The benefit of fNIRS (compared to fMRI) lies in its ease of use, low cost, ecological validity (no noise, comfortable measurements in a more natural environment), and relatively low susceptibility to motion artifacts. Thus, fNIRS may be particularly suited to investigate neural functions in infants and in psychiatric patients. On the other hand, fNIRS has a relatively poor spatial resolution and is restricted to cortical measurements, which can be affected by confounding influences such as individual anatomy or systemic physiological artifacts decreasing the signal to noise ratio (Cui et al., 2011; Hoshi, 2007).

Imaging Genetics

To investigate whether a particular genotype has a phenotypic effect on the level of neural processing/activation or neuroanatomy as assessed with functional neuroimaging, these measures are statistically investigated for differences between genotype groups of subjects. This approach is referred to as Imaging Genetics or Genomic Imaging (first described by: Fallgatter et al., 1999). Genetic effects show higher penetrance to the level of neural activation or anatomy compared to effects on behavioral phenotypes or psychopathology. Thereby, Imaging Genetics may provide insight into the genetic underpinnings of neural processing or pathomechanisms of psychiatric illnesses (Gottesman and Gould, 2003).

Each functional neuroimaging method has its own merits and limitations, and by using different modalities as well as functional tasks, the three studies included in the present cumulative dissertation yield different perspectives of prefrontal cognitive

control and executive functions, their neural correlates and aspects of methodological approaches in functional neuroimaging.

In Study #1 EEG and P300 ERP recordings were used to examine prefrontal cognitive response control during a response inhibition task in a sample of healthy controls and adult ADHD patients. The neural and behavior correlates of cognitive response control, which were hypothesized to differ between these groups, were furthermore investigated for an (epistatic) impact of two dopaminergic gene variants associated with altered PFC processing and ADHD, respectively.

In Study #2 cortical hemodynamic responses elicited by prefrontal cognitive/executive processing underlying verbal fluency were measured in elderly subjects using fNIRS. The impact of age, sex, years of education and task performance on the hemodynamic correlates of cortical verbal fluency processing was examined as part of a multidisciplinary longitudinal study aiming to identify risk factors of neurodegeneration in Alzheimer's disease and Parkinson's disease, respectively.

In Study #3 simultaneous fNIRS-fMRI measurements were used to (1) compare these neuroimaging methods regarding prefrontal hemodynamic responses during an intertemporal reward choice task, (2) investigate individual anatomical and systemic physiological factors impacting fNIRS measurements, and (3) to examine the correlation between trait "sensitivity to reward" and the fNIRS and fMRI activation measures, respectively, and the impact of sources of error variance on activation-trait associations in fNIRS data.

While the details of these studies were not in the scope of this general introduction, the specific background, hypotheses and methods used to investigate PFC (dys)function are given in the respective sections of the following publications.

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Manuscript:

"*COMT* x *DRD4* epistasis impacts prefrontal cortex function underlying response control"

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COMT × *DRD4* Epistasis Impacts Prefrontal Cortex Function Underlying Response Control

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The prefrontal cortex plays a major role in cognitive control, but it is unclear how single genes and gene–gene interactions (genetic epistasis) impact neural and behavioral phenotypes. Both dopamine (DA) availability ("inverted U-model") and excitatory versus inhibitory DA receptor stimulation ("dual-state theory") have been linked to important principles of prefrontal processing. Catechol-*O*-methyltransferase (*COMT*; Val158Met) and DA D4-receptor (*DRD4*; 48 bp VNTR) genotypes were analyzed for effects on behavioral and neural correlates of prefrontal response control (NoGo-anteriorization, NGA) using a Go–NoGo task and electroencephalography (114 controls and 181 patients with attention-deficit/hyperactivity disorder). *DRD4* and *COMT* epistatically interacted on the NGA, whereas single genes and diagnosis showed no significant impact. Subjects with presumably relatively increased D4-receptor function (*DRD4*: no 7R-alleles) displayed an inverted U-relationship between the NGA and increasing *COMT*-dependent DA levels, whereas subjects with decreased D4-sensitivity (7R) showed a U-relationship. This interaction was supported by 7R-allele dose effects and mirrored by reaction time variability (non-significant after multiple testing correction). Combining previous theories of prefrontal DA functioning, neural stability at intermediate DA levels may be accompanied by the risk of overly decreased neural flexibility if inhibitory DA receptor function is additionally decreased. Our findings might help to disentangle the genetic basis of dopaminergic mechanisms underlying prefrontal (dys)function.

Keywords: attention-deficit/hyperactivity disorder (ADHD), dopamine, electroencephalography, genetic epistasis, NoGo-anteriorization

Introduction

Dysfunction of the prefrontal cortex (PFC) has been implicated in the etiology of psychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD) or schizophrenia. While these neuropsychiatric disorders are highly heritable (Sullivan et al. 2003; Faraone et al. 2005), it is largely unclear how putative genetic risk factors affect PFC processing and behavioral phenotypes. Furthermore, due to the complex multifactorial genetic architecture of these disorders, individual risk alleles contribute only small effects to pathological symptoms [ADHD: pooled odds ratio (OR): 1.2–1.4 (Gizer et al. 2009)]. Moreover, genetic epistasis, that is, an interaction of non-allelic (multiple locus) genes on phenotypic variation, may modulate neural mechanisms of PFC processing (Marchini et al. 2005).

Current influential neural models of dopamine (DA) function within the PFC suggest that both DA levels and the ratio of stimulation of excitatory (D1-like receptor subfamily: D1 and D5) and inhibitory (D2-like: D2, D3, and D4) DA receptors play a pivotal role for PFC processing and functional outcome.

Specifically, (1) an inverted U-shaped relation between prefrontal DA levels and brain activation and task performance during cognitive control processes, as involved in working memory, has been repeatedly reported (for recent reviews, see Arnsten 2011; Cools and D'Esposito 2011). Moderate levels of D1-receptor stimulation have been shown to mediate stabilization of neural representations, neural tuning, and decrease in noise optimal for the online maintenance of information within the PFC (Seamans and Yang 2004; Vijayraghavan et al. 2007). However, too little or too much DA or D1-receptor stimulation, respectively, may be disruptive and impair these prefrontal processing features (Arnsten and Goldman-Rakic 1998; Bilder et al. 2004; Vijayraghavan et al. 2007).

Yet, neurobehavioral processes, such as cognitive control, are of multifactorial nature, often requiring both stable and flexible network characteristics (Cools and D'Esposito 2011). In this regard, (2) the "dual-state theory" postulates differential DA receptor type stimulation to underlie distinct energy barriers among prefrontal neural network patterns (Durstewitz and Seamans 2008). While a D1-dominated (high energy) network state favors cognitive stability, the D2-dominated state (low energy) is associated with cognitive flexibility among neural representational states.

Thus, depending on the nature of the task components, an imbalanced ratio of D1-like to D2-like receptor stimulation may bias prefrontal processing dynamics affecting cognitive performance, which may partly underlie deficits in attention and executive function in ADHD and schizophrenia (Durstewitz and Seamans 2008; Kehrer et al. 2008; Rolls et al. 2008). However, the precise interaction between DA levels and DA receptor type stimulation on prefrontal processing is still largely unclear. Thus, investigating prefrontal processes with regard to the impact and (epistatic) interaction of these 2 factors, as indicated by functional gene variants, may shed light on the (dys)regulation of dopaminergic processing underlying prefrontal functions such as cognitive control. Two candidate genes, which we hypothesized to exert such epistatic effects on prefrontal processing and behavioral

outcome, code for catechol-*O*-methyltransferase (*COMT*) and the DA D4-receptor (*DRD4*).

The enzymatic degradation of DA by COMT (Karoum et al. 1994) is influenced by a single nucleotide polymorphism (SNP; Val158Met) causing a substitution of valine (Val) with methionine (Met) resulting in 3–4-fold reduced enzymatic activity and, therefore, increased baseline synaptic DA (Chen et al. 2004). In healthy subjects, prefrontal function is modulated by *COMT*-dependent DA levels, which maps to an inverted U-curve with best working memory performance at intermediate DA levels (Meyer-Lindenberg et al. 2005). However, genetic associations of Val158Met with ADHD diagnosis have been inconsistent (Gizer et al. 2009).

DA binding to D4-receptors (D2-like type) exerts inhibitory effects on neuronal and PFC activity (Yuen and Yan 2009) balancing GABAergic inhibition and glutamatergic excitation via precise tuning mechanisms (Seamans, Gorelova et al. 2001). D4-receptor sensitivity has been shown to differ between the *DRD4* variants, 4-repeats (4R) and 7-repeats (7R) of 48 bp, respectively. The 7R variant has a 2-fold decreased D4-receptor sensitivity compared with 4R (Asghari et al. 1995). Decreased D4-receptor function has been linked to impulsivity and novelty seeking (Ebstein et al. 1996; Avale et al. 2004), and the 7R-allele has been shown to be associated with ADHD [OR: 1.33, 1.15–1.54; (Gizer et al. 2009)].

The present study used an "Imaging Genetics" approach (first described in Fallgatter, Jatzke et al. 1999) to investigate statistical main effects and *COMT* × *DRD4* epistasis on neurophysiological and behavioral correlates of cognitive response control in healthy controls and adult patients with ADHD.

Cognitive response control is one of the frontal lobe executive functions frequently disturbed in ADHD that has been suggested as a neurocognitive endophenotype of the disorder (Slaats-Willemse et al. 2003). We used a reliable endophenotypic marker of prefrontal functioning, reflecting neural correlates of both response inhibition and execution in a Go–NoGo test situation [NoGo-anteriorization (NGA; Fallgatter and Strik 1999)]. The NGA is a topographic event-related potential (ERP) parameter quantifying the brain's electrical field frontalization during motor inhibition (NoGo, when compared with response execution: Go). Validated as a neurophysiological index of cognitive response control, the NGA reflects "NoGo" activation of the medial PFC (anterior cingulate cortex, ACC) (Fallgatter et al. 2002). The NGA and the electrical field frontalization during NoGo trials were shown to be reduced in schizophrenia and ADHD compared with healthy controls, reflecting diminished activation of the medial PFC in these patients (Fallgatter and Muller 2001; Fallgatter et al. 2003, 2005). Behavioral Go–NoGo performance can be quantified by the mean Go reaction time (Go-RT), its intra-individual variability (i.e. standard deviation, Go-RT SD), as well as response error rates. Particularly an increased Go-RT SD has been shown in patients with ADHD and linked to altered catecholaminergic processing (Castellanos et al. 2005). Functionally, increased mean Go-RTs as well as Go-RT SD may, as an adaptation strategy, allow for a decrease in response error rates; alternatively, an increased Go-RT SD has been discussed to reflect lapses in attention or an impaired "state regulation", possibly due to cortical under-arousal (Klein et al. 2006).

Endophenotypic differences in cognitive response control (Sonuga-Barke 2002), decreased D4-receptor sensitivity (Faraone et al. 2005), and catecholaminergic dysfunction (Prince 2008) have been implicated in the pathophysiology of ADHD. Motivated by these findings, we investigated differences in genetic main and epistatic effects on neural and behavioral response control between healthy controls and ADHD patients. In order to determine the universality of our findings, we investigated a combined sample of healthy controls—with theoretically "normal" PFC DA metabolism—and ADHD patients, with putatively deviant PFC DA metabolism and an assumed DA-dependent dysregulation of PFC function.

Previously, our group showed gene main effects of tryptophan hydroxylase 2 (*TPH2*) and DA transporter (*DAT*) on the NGA and Go and NoGo electrical fields, respectively, in healthy controls and adult ADHD patients (Baehne et al. 2009; Dresler et al. 2010). While adjusting our statistical tests to accommodate for this multiple testing situation, the present study aimed to investigate the hypothesis of *COMT* × *DRD4* epistasis on neural and behavioral response control. We hypothesized that reduced neural flexibility due to decreased D4-receptor function (*DRD4* 7R genotype) would impact cognitive response control depending on prefrontal DA levels, specifically in the presence of intermediate DA levels/D1-stimulation (*COMT* Val/Met genotype) fostering rigid and stable neural representations.

Materials and Methods

Participants

One hundred and eighty one adult ADHD patients and 114 healthy controls without history of psychiatric or neurological disorders were included in the study. ADHD patients as well as healthy controls were diagnosed by experienced psychiatrists using the Structured Clinical Interview for DSM-IV (SCID-I) (Wittchen et al. 1997). Participants were of Caucasian origin and recruited via the in- and outpatient facilities of the Department of Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg. All participants gave written informed consent. Patients and controls were currently not taking any psychotropic medication (89.5% of patients were naive for ADHD medication and 10.5% were off medication for at least 3 days prior to testing). Further exclusion criteria were age below 18 and above 60 years and IQ below 90. The sample partly overlaps with 2 of our previous studies (Baehne et al. 2009; Dresler et al. 2010).

All participants were stratified according to the *COMT* Val158Met genotype (Val/Val, Val/Met, and Met/Met) and *DRD4* 48 bp VNTR genotype [no 7 repeat allele (no 7R), at least one 7 repeat allele (7R)]. Genotype frequencies and descriptive statistics are displayed in Table 1. *DRD4* genotype frequencies did not differ between diagnostic groups ($\chi^2_1 = 0.77$, $P = 0.38$), and allele frequencies were in Hardy–Weinberg equilibrium (HWE) (controls: $\chi^2_1 = 0.73$, $P = 0.69$ and ADHD: $\chi^2_1 = 0.01$, $P = 0.93$). *COMT* genotype frequencies did not differ between diagnostic groups ($\chi^2_2 = 0.73$, $P = 0.69$). *COMT* Val158Met allele frequencies were in HWE for controls ($\chi^2_1 = 0.55$, $P = 0.46$), but not for ADHD patients ($\chi^2_1 = 4.01$, $P = 0.045$).

Handedness, age distribution, and gender ratios did not differ between diagnostic groups or genotypes ($P > 0.1$). IQ scores were assessed using the MWT-B (Lehrl 2005), which measures crystallized verbal intelligence (for 2 patients no IQ data were available). For IQ scores, a $2 \times 2 \times 3$ analysis of variance (ANOVA) yielded a significant effect for diagnosis ($F_{1,272} = 15.83$, $P < 0.01$, $\eta^2 = 0.055$), with higher scores for controls. ADHD patients had higher scores on the retrospective assessment of ADHD symptom severity in childhood [Wender Utah Rating Scale (WURS-k)] (Retz-Junginger et al. 2002) ($F_{1,276} = 189.53$, $P < 0.001$, $\eta^2 = 0.407$; for 7 patients the WURS-k score was missing). *COMT* and *DRD4* genotype, epistatic interactions, or interactions with diagnosis had no significant influence on WURS-k scores. 65.5% of the patients were diagnosed with the combined, 26.0% with the inattentive, and 8.5% with the hyperactive/

Table 1
Sample characteristics (standard deviation in parentheses)

ADHD ($n = 181$)

COMT genotypes	No 7R ($n = 105$)			7R ($n = 76$)			
	Val/Val ($n = 25$)	Val/Met ($n = 44$)	Met/Met ($n = 36$)	Val/Val ($n = 25$)	Val/Met ($n = 33$)	Met/Met ($n = 18$)	All ($n = 181$)
Age (years)	34.08 (9.33)	36.86 (9.47)	35.89 (10.51)	35.00 (9.25)	34.15 (10.08)	35.61 (10.60)	35.48 (9.78)
IQ	110.96 (10.71)	111.81 (13.26)	110.53 (12.49)	115.65 (15.63)	114.33 (10.54)	108.29 (13.75)	111.96 (12.66)
Handedness (right/left)	24/1	42/2	33/3	24/1	28/5	15/3	166/15
Female (%)	48.0	43.2	41.7	68.0	48.5	50.0	48.6
WURS-k	32.33 (14.49)	39.14 (14.16)	34.31 (12.42)	34.22 (10.92)	36.72 (14.91)	40.00 (16.26)	36.22 (13.90)

Controls ($n = 114$)

COMT genotypes	No 7R ($n = 72$)			7R ($n = 42$)			
	Val/Val ($n = 16$)	Val/Met ($n = 37$)	Met/Met ($n = 19$)	Val/Val ($n = 16$)	Val/Met ($n = 16$)	Met/Met ($n = 10$)	All ($n = 114$)
Age (years)	32.25 (10.82)	36.11 (9.39)	34.26 (11.58)	37.75 (9.83)	33.37 (9.77)	40.70 (11.28)	35.51 (10.31)
IQ	115.19 (14.57)	117.40 (12.59)	121.06 (12.72)	122.00 (9.96)	114.19 (13.84)	121.00 (15.73)	118.10 (13.08)
Handedness (right/left)	13/3	33/4	18/1	15/1	15/1	9/1	103/11
Female (%)	56.3	59.5	57.9	31.3	62.5	30.0	52.6
WURS-k	13.44 (6.93)	12.13 (8.15)	11.63 (9.18)	14.81 (10.77)	15.75 (7.65)	15.40 (10.24)	13.41 (8.65)

impulsive subtype of ADHD (for 8 patients, subtype diagnosis was missing). Subtype distribution did not differ between *DRD4* and *COMT* genotype groups ($P > 0.1$).

49.2% of the ADHD patients had a current psychiatric comorbid axis I disorder as evaluated with the SCID-I (see Supplementary Material for further details). Furthermore, 82 ADHD patients (44.3%) and 19 controls (16.7%) were daily tobacco smokers ($\chi^2_1 = 16.49$, $P < 0.001$).

The study was in accordance with the latest version of the Declaration of Helsinki and approved by the Ethics Committee of the University of Würzburg.

Genotyping

Genomic DNA was extracted from whole blood. Genotyping was performed as described previously for *DRD4* 48 bp VNTR (Ebstein et al. 1996) and *COMT* Val158Met (Egan et al. 2001), respectively, using polymerase chain reaction, enzymatic digestion, and gel electrophoresis. Further details on protocols are available upon request.

Electrophysiological Investigation

The electroencephalography (EEG) experiment was a Go–NoGo task (an OX version of the continuous performance test), which requires participants to respond or to inhibit button responses to sequentially presented letter stimuli. Specifically, participants had to quickly respond when the letter O was directly followed by the letter X and to otherwise inhibit the response. Performance speed and accuracy were emphasized equally in the task instruction. After a short training session, the task comprised 400 letters (114 O = primer condition, 57 X following an O = Go condition, 57 other letters following an O = NoGo condition, and 172 letters not following an O = distractors), each presented for 200 ms with a stimulus-onset asynchrony of 1850 ms (total duration of the task: 13 min).

During the Go–NoGo task, a continuous EEG was recorded from 21 scalp electrodes placed according to the International 10/20 System. EEG signals were amplified by a 32-channel DC amplifier and recorded using "Vision Recorder" (Brain Products, Munich, Germany) with a bandpass filter (0.1–100 Hz) and 1000 Hz A/D rate. The recording reference was placed between Fz and Cz and the ground electrode between Fpz and Fz. All electrode impedances were kept below 5 kΩ.

Data Analysis

Electrophysiological data analyses were conducted using "Vision Analyzer" (Brain Products). After offline bandpass filtering (0.1–70 Hz), data were re-referenced to an average reference and corrected for artifacts (Supplementary Material). Artifact-free epochs with correct behavioral responses were segmented and averaged to Go and NoGo ERPs. Two-dimensional area centroids of P300 field maps (amplitude-weighted "centers of gravity" of the brain electrical field) were calculated for Go and NoGo conditions using individual P300 latencies at Pz (Go) and Cz (NoGo), respectively; P300 peaks were defined as the most positive deflection within 275–530 ms poststimulus. Individual centroids were localized on an anterior–posterior axis of a coordinate system resulting from the planar projection of the electrode array onto a rectangular grid. Positional centroid values were between 1 (Fpz) and 5 (Oz), and thus smaller values indicate a more anterior localization. Finally, the NGA was calculated individually as the difference between Go and NoGo centroids (Fallgatter and Strik 1999). Additionally, baseline-corrected Go and NoGo P300 peaks were exported for further (traditional) ERP analyses (baseline period: −100 to 0 ms pre-stimulus).

Source Localization

In order to identify brain regions significantly contributing to NGA differences between genotype groups, we utilized the standardized low-resolution brain electromagnetic tomography (sLORETA) software (http://www.uzh.ch/keyinst/loreta.htm) (Pascual-Marqui 2002). sLORETA is a weighted minimum norm inverse solution used to compute statistical maps from scalp potentials, indicating the location of underlying neural sources (Pascual-Marqui et al. 2002). Voxel-based sLORETA images were compared between 6 genotype groups (combined sample) using time points of maximal differences in scalp topography (Ehlis et al. 2011). Genotype group differences between these maps were then statistically analyzed.

Statistical Analysis

Statistical analyses were performed using SPSS 17.0. Since the present sample largely overlaps with 2 of our previous studies investigating main effects of 2 SNPs within the *TPH2* gene (Baehne et al. 2009) and 1 variable number of tandem repeats polymorphism of the *DAT* (*SCL6A3*; *DAT*) gene (Dresler et al. 2010), we used a Bonferroni correction for multiple testing considering the 5 investigated genetic polymorphisms (including *COMT* and *DRD4*). Therefore, the significance level was set to $P < 0.01$ for analyses of gene main and epistasis effects on neural (NGA) and behavioral measures. For analyses of genotypes impacting NGA and behavioral measures (mean Go-RT and Go-RT SD), $2 \times 2 \times 3$ ANOVAs were conducted, comprising the between-subject factors "diagnosis," "*DRD4*," and "*COMT*." For conventional analysis of P300 amplitudes, similar ANOVAs were applied, additionally comprising the within-subject factors "condition" (Go vs. NoGo) and "electrode position" (Cz and Pz). Since post hoc analyses aimed to identify effects already indicated by ANOVA models corrected for comparison of multiple gene variants, post hoc tests and (exploratory) sLORETA tests were calculated using 2-tailed *t*-tests for independent samples, which were uncorrected for multiple testing ($P < 0.05$). Equality of variances was tested by means of Levene's test. According to Kolmogorov–Smirnov's *Z*-statistic, all data were normally distributed except Go–NoGo task performance error data ($P < 0.01$), for which Mann–Whitney *U* tests and Kruskal–Wallis tests were applied for between-group comparisons. Tests for quadratic relationships were performed using polynomial trend tests ($P < 0.05$).

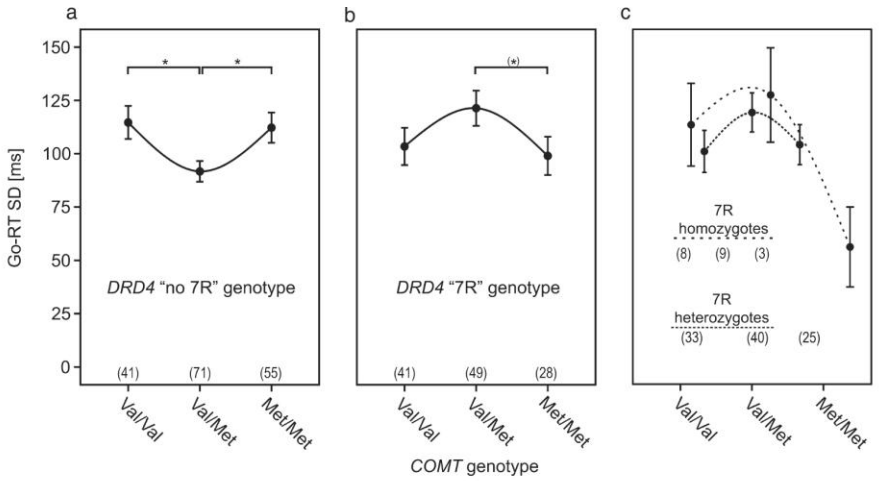

Figure 1. (*a*) Intra-individual variability in Go-RT (Go-RT SD) in *COMT* genotype subgroups of *DRD4* "no 7R"-carriers of the combined sample (ADHD patients and controls). The spline curve through mean Go-RT SD values displays the quadratic relationship of Go-RT SD between *COMT* subgroups. (*b*) Go-RT SD distributions by *COMT* genotype in *DRD4* "7R"-carriers (*c*) Go-RT SD distributions by *COMT* genotype separated into *DRD4* 7R homozygotes and heterozygotes. Error bars indicate standard error of the mean (SEM). Asterisks and asterisks in brackets indicate significant differences at a significance level of $P < 0.05$ and $P < 0.10$, respectively. Numbers in brackets indicate size of genotype subgroups.

Results

Behavioral Data

The ANOVA of Go-RT and Go-RT SD yielded a significant main effect for diagnosis. ADHD patients had marginally significantly longer Go-RT ($F_{1,283} = 6.04$, $P = 0.015$, effect size $\eta^2 = 0.021$) and increased Go-RT SD ($F_{1,283} = 14.64$, $P < 0.001$, $\eta^2 = 0.049$) compared with controls. Additionally, patients made more omission errors ($U = 8044.0$, $Z = -3.46$, $P < 0.01$) and commission errors (type 1) after primers and distractors ($U = 7858.0$, $Z = -3.81$, $P < 0.01$). The number of successful response inhibitions during NoGo trials (commission error type 2) did not differ between controls and ADHD patients ($U = 10\,247.5$, $Z = -0.181$, $P > 0.1$).

No main effects of *DRD4* or *COMT* genotype on Go-RT and Go-RT SD were observed ($P > 0.1$). Epistatic interactions of *DRD4* × *COMT* on Go-RT ($F_{2,283} = 3.13$, $P = 0.045$, $\eta^2 = 0.022$) and Go-RT SD ($F_{2,283} = 3.62$, $P = 0.028$, $\eta^2 = 0.025$; Fig. 1*a*,*b*) were numerically present, but did not reach the predefined significance level after correcting for multiple testing. Also, ADHD diagnosis did not explain further variance of gene main or epistatic effects ($P > 0.3$) (for separate analyses of diagnostic groups, see Supplementary Material). The number of *COMT* Met alleles and Go-RT SD followed quadratic relationships depending on *DRD4* genotype. Subjects (combined sample) without 7R-alleles displayed a U-relationship ($F_{1,174} = 8.91$, $P = 0.003$), whereas 7R-carriers showed a marginally significant inverted U-relationship ($F_{1,115} = 3.79$, $P = 0.054$). Exploratory analyses of *DRD4* 7R-allele dose effects on Go-RT SD supported this finding. Descriptively, homozygous carriers of *DRD4* 7R showed a more pronounced inverted U-shape than 7R heterozygotes. Specifically, 7R homozygotes with *COMT* Met/Met genotype ($n = 9$) exhibited decreased Go-RT SD compared with corresponding 7R heterozygotes ($n = 40$) (Fig. 1*c*).

DRD4 genotype had no influence on omission or commission errors ($P > 0.1$). *COMT* genotype had no impact on commission errors ($P > 0.1$), but a significant effect on omission errors in controls ($\chi^2_2 = 11.75$, $P = 0.003$). Here, Val/Met carriers made less omission errors compared with Val/Val ($U = 530.0$, $P = 0.001$) but not with Met/Met ($U = 598.5$, $P = 0.037$) carriers, whereas Val/Val and Met/Met did not differ significantly ($U = 401.5$, $P > 0.1$; Table 2).

Table 2
Descriptive statistics of behavioral data (standard deviation in parentheses)

ADHD ($n = 181$)

COMT genotypes	No 7R ($n = 105$)			7R ($n = 76$)			
	Val/Val ($n = 25$)	Val/Met ($n = 44$)	Met/Met ($n = 36$)	Val/Val ($n = 25$)	Val/Met ($n = 33$)	Met/Met ($n = 18$)	All ($n = 181$)
Commission errors type 1	1.28 (1.28)	1.18 (1.67)	1.14 (1.97)	1.12 (2.42)	0.97 (1.16)	0.61 (0.70)	1.13 (1.74)
Commission errors type 2	0.04 (0.20)	0.25 (0.72)	0.03 (0.17)	0.16 (0.37)	0.33 (0.92)	0.28 (1.18)	0.19 (0.67)
Omission errors	1.60 (2.96)	2.09 (2.96)	2.36 (3.90)	1.64 (2.69)	2.48 (3.18)	1.11 (2.08)	1.98 (3.04)
Go-RT	510.38 (104.74)	484.13 (117.37)	507.06 (142.91)	485.41 (123.07)	539.21 (134.59)	460.84 (90.45)	498.21 (122.57)
Go-RT SD	116.70 (45.39)	98.73 (49.14)	120.94 (53.05)	113.18 (59.90)	139.37 (56.91)	105.62 (49.62)	115.02 (53.09)

Controls ($n = 114$)

COMT genotypes	No 7R ($n = 72$)			7R ($n = 42$)			
	Val/Val ($n = 16$)	Val/Met ($n = 37$)	Met/Met ($n = 19$)	Val/Val ($n = 16$)	Val/Met ($n = 16$)	Met/Met ($n = 10$)	All ($n = 114$)
Commission errors type 1	0.42 (0.77)	0.49 (0.84)	0.88 (1.03)	0.38 (1.03)	0.44 (0.51)	0.10 (0.32)	0.47 (0.82)
Commission errors type 2	0.21 (0.54)	0.14 (0.42)	0.13 (0.34)	0.19 (0.40)	0.00 (0.00)	0.10 (0.32)	0.13 (0.39)
Omission errors	1.05 (1.65)	0.65 (1.78)	1.25 (1.69)	1.44 (1.59)	0.25 (0.45)	1.10 (1.66)	0.89 (1.60)
Go-RT	493.66 (117.63)	445.09 (102.30)	480.60 (145.66)	466.04 (120.34)	456.94 (88.93)	421.27 (77.49)	460.34 (111.56)
Go-RT SD	111.46 (57.28)	83.36 (35.23)	95.67 (48.40)	88.13 (46.59)	84.13 (40.80)	87.01 (43.81)	90.45 (44.24)

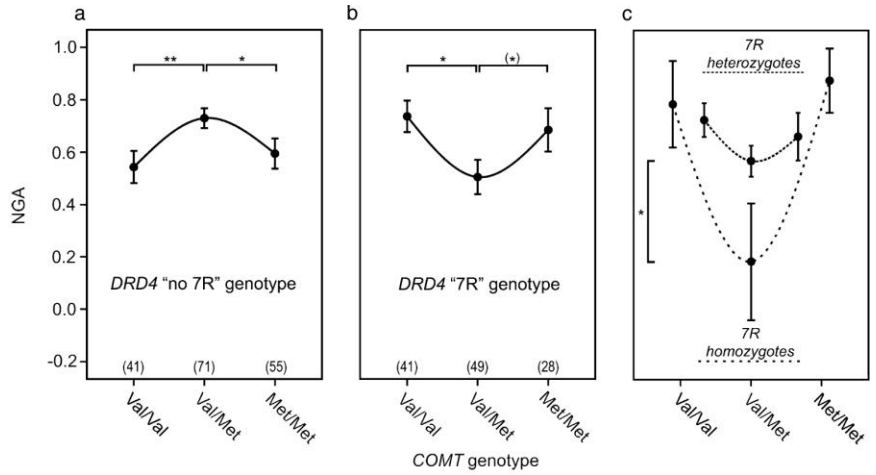

Figure 2. (*a*) NGA in *COMT* genotype subgroups of *DRD4* "no 7R"-carriers of the combined sample (ADHD patients and controls). (*b*) NGA distributions by *COMT* genotype in *DRD4* "7R"-carriers. (*c*) NGA distributions by *COMT* genotype separated into *DRD4* "7R" homozygotes and heterozygotes. Error bars indicate SEM. Numbers in parentheses indicate size of genotype subgroups.

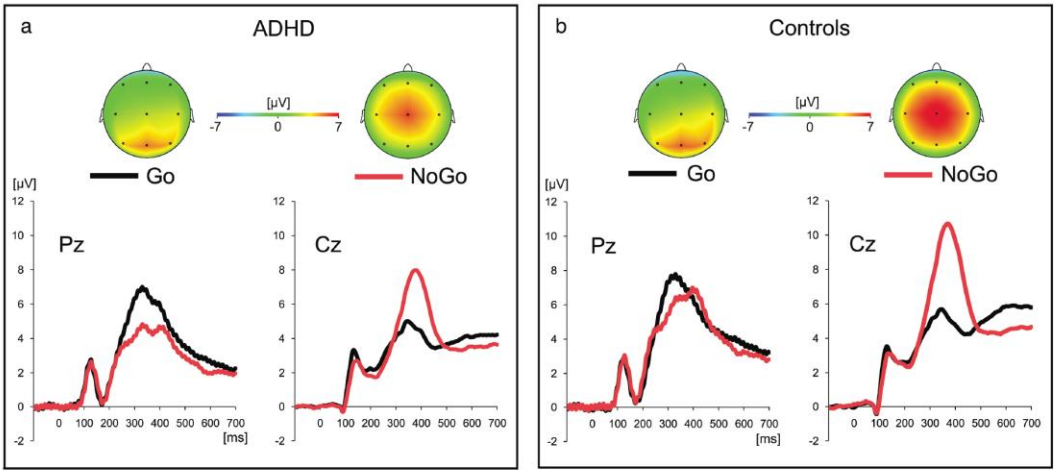

Figure 3. P300 topography and ERPs for Go and NoGo conditions at Cz and Pz electrode positions in (*a*) ADHD patients and (*b*) healthy controls.

ERP Data

NGA did not differ between ADHD patients and controls ($F_{1,283} = 0.56$, $P = 0.46$; ADHD: 0.62 ± 0.43 and controls: 0.67 ± 0.36; mean ± SD). Furthermore, no significant main effects of *DRD4* and *COMT* genotype on the NGA were observed (*DRD4*: $F_{1,283} = 0.10$, $P = 0.76$ and *COMT*: $F_{2,283} = 0.06$, $P = 0.94$). However, *DRD4* × *COMT* showed a significant interaction ($F_{2,283} = 6.21$, $P = 0.002$, $\eta^2 = 0.042$; Fig. 2*a*,*b*). Subjects lacking the *DRD4* 7R-allele exhibited an inverted U-relationship between the number of *COMT* Met alleles and the NGA ($F_{1,174} = 7.78$, $P = 0.006$), whereas *DRD4* 7R-allele carriers showed a marginally significant U-relationship ($F_{1,115} = 6.49$, $P = 0.012$). As diagnosis did not significantly impact the *DRD4* × *COMT* interaction, its shape was similar in ADHD patients and controls (Supplementary Material). Post hoc analyses showed that *DRD4* genotype impacted NGA in Val/Met ($t_{128} = 3.20$, $P = 0.002$, Cohen's $d = 0.56$) and Val/Val ($t_{80} = -2.26$, $P = 0.03$, $d = 0.51$), but not in Met/Met carriers ($t_{81} = -0.90$, $P = 0.37$, $d = 0.21$) in the combined sample.

The profound impact of *DRD4* and *COMT* epistasis on NGA was additionally supported by 7R-allele dose effects. Descriptively, in homozygous carriers of *DRD4* 7R, the NGA followed a more pronounced U-relationship with increasing Met alleles compared with 7R heterozygotes (Fig. 2*c*). In *COMT* Val/Met carriers, *DRD4* 7R homozygotes showed a significantly decreased NGA compared with 7R heterozygotes ($t_{47} = 2.46$, $P = 0.018$, d = 0.93).

Statistical results of genetic main and interaction effects on the NGA and behavioral measures remained largely unchanged after introducing "age" and "gender" as covariates in the analysis, as we expected from previous findings (Fallgatter, Mueller et al. 1999).

Complementing our findings of the genetic impact on the relative measure of the NGA, traditional ERP waveforms of Go and NoGo trials, respectively, as well as the corresponding topography, are shown for controls and ADHD patients in Figure 3*a*,*b*. Supporting findings of *COMT* × *DRD4* epistasis on the NGA, analyses of the Go and NoGo P300 amplitudes at

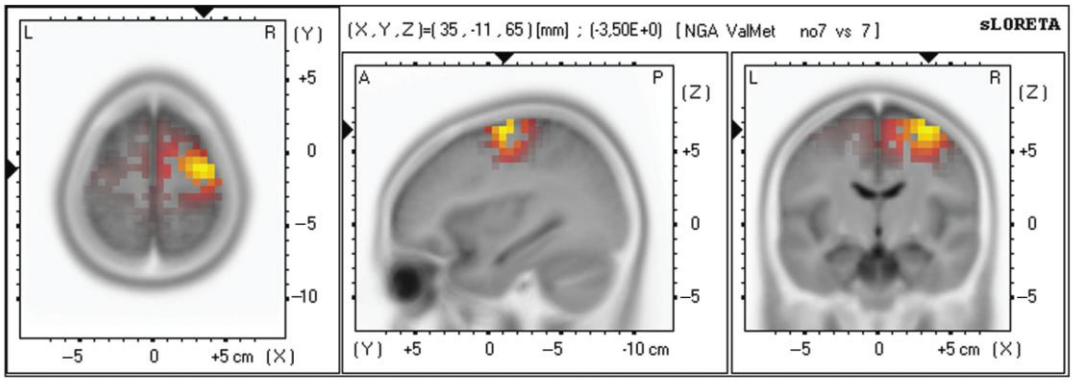

Figure 4. Source localization with sLORETA shows the NGA (NoGo–Go) contrast for *DRD4* "7R" versus "no 7R" within the *COMT* Val/Met subgroup of the combined sample. *DRD4* genotype-dependent differences in NGA are mapped to Brodmann area 6 (right precentral gyrus).

electrode positions Cz and Pz also showed a significant (at $P<0.05$, i.e. uncorrected for multiple testing) 4-fold interaction ($F_{2,283}=3.24$, $P=0.04$) between conditions (Go and NoGo), positions (Cz and Pz), *DRD4* (7R and no 7R), and *COMT* (0, 1, and 2 Met alleles). Post hoc tests revealed that, for instance, "no 7R"-carriers exhibited an inverted U-relationship between P300 amplitudes and the number of *COMT* Met alleles (most pronounced for the NoGo condition and position Cz: $F_{1,174}=4.16$, $P=0.04$, quadratic trend test), which exactly mirrors the finding reported previously for the NGA. In contrast to that, for the group of 7R-carriers, the corresponding test showed a rather linear relationship ($F_{1,115}=2.77$, $P=0.099$; linear trend test). Again, similar to the NGA data, diagnosis had no additional impact on these genetic interactions ($P>0.1$). However, (1) compared with controls, ADHD patients generally showed significantly lower P300 amplitudes in both task conditions at both positions (main effect diagnosis: $F_{2,283}=19.36$, $P<0.01$); (2) the reduction in P300 amplitude from Go to NoGo at Pz was more pronounced in ADHD patients (1.59 ± 2.49 μV) compared with controls (0.82 ± 2.83 μV; $t_{293}=2.45$, $P<0.05$); whereas (3) the amplitude increase from Go to NoGo at Cz was more pronounced in controls (4.61 ± 3.55 vs. 2.64 ± 3.14 μV; $t_{293}=4.97$, $P<0.001$) underlying the interaction of condition × position × diagnosis ($F_{1,283}=8.63$, $P=0.004$; see also Fig. 3*a*,*b*).

P300 amplitudes were negatively correlated with behavioral measures (Go trials at Pz: Go-RT: $r=-0.34$, Go-RT SD: $r=-0.27$ and NoGo trials at Cz: Go-RT: $r=-0.41$, Go-RT SD: $r=-0.52$; $P<0.001$). NoGo-P300 values remained highly significantly correlated with behavioral measures in the 6 genotype subgroups, whereas correlations of Go-P300 values with Go-RT and Go-RT SD ranged between $r=-0.12$ (not significant) and $r=-0.40$ ($P<0.001$) in the six genotype subgroups. For the NGA, smaller negative associations with behavioral measures were observed (Go-RT: $r=-0.12$, $P=0.04$ and Go-RT SD: $r=-0.11$, $P=0.07$).

Source Localization

Source localization revealed Brodmann area 6 (precentral gyrus) to significantly contribute to differences in NGA comparing *DRD4* genotype groups in *COMT* Val/Met carriers ($t_{128}=3.84$, $P=0.013$; Montreal Neurological Institute coordinates: $x=35$, $y=-11$, $z=40$; Fig. 4) and comparing *COMT* Val/Met with Val/Val genotypes within *DRD4* no 7R-carriers ($t_{120}=3.98$, $P=0.014$; supplementary motor cortex; $x=25$, $y=-5$, $z=70$). All other source localizations using genotype group comparisons did not reach statistical significance.

Discussion

The present data provide evidence for an epistatic interaction of *DRD4* × *COMT* on neurophysiological correlates of prefrontal function underlying cognitive response control in adult ADHD patients and healthy controls. Prefrontal response control as indicated by the anterior shift of the brain electrical field during NoGo relative to Go trials (NGA) followed (inverted) U-shapes with increasing *COMT*-dependent DA levels, depending on *DRD4* genotype. Genotype-dependent effects on the NGA could be localized in an explorative analysis to the right premotor and supplementary motor area. Mirroring the neural effects, *COMT* × *DRD4* epistasis showed similar (inverted) U-effects on the behavioral level (Go-RT and Go-RT SD), which were, however, no longer significant after strictly correcting for multiple testing. These results suggest that the function of D4-receptors depending on prefrontal DA levels has a profound impact on PFC processing. Moreover, the present results confirm our hypothesis that a genotype-dependent dysbalance of inhibitory (D4) and excitatory (D1; intermediate DA levels) DA receptor stimulation would decrease cognitive control function as indicated by the NGA and Go-RT SD. ADHD diagnosis had no significant impact on these epistatic interactions, and our findings may, thus, reflect basic principles of the genetic and dopaminergic regulation of PFC processing. The findings are discussed regarding the inverted U-model and the dual-state theory of prefrontal DA function as well as possible underlying neurobiological mechanisms.

Commonly, effects of varying DA levels have been investigated regarding actions of D1-receptors. For instance, D1-receptors have been shown to increase activity of PFC pyramidal neurons by enhancing *N*-methyl-D-aspartate (NMDA) receptor postsynaptic currents (Seamans, Durstewitz et al. 2001; Wang and O'Donnell 2001) and to increase feedforward GABAergic inhibition of pyramidal neurons reducing background activity (Durstewitz et al. 2000; Durstewitz and Seamans 2002). This may partly explain increasing signal-to-noise ratio (SNR) with increasing *COMT*-dependent DA levels (Winterer et al. 2006). Furthermore, for both DA concentration and D1-

receptor stimulation, an inverted U-shaped response function has been proposed, according to which too little or too much DA or D1-receptor stimulation, respectively, is disruptive and impairs functioning of the system (Arnsten and Goldman-Rakic 1998; Bilder et al. 2004; Vijayraghavan et al. 2007).

As a basis for the dual-state theory, effects of DA depend on the relative amount and efficiency of different DA receptor classes in target regions. Thus, the dual-state theory represents an extension of the inverted U-shaped model, which focusses on D1-stimulation and DA levels, respectively. While the precise mechanisms are not yet completely understood, DA-concentration dependency of D2-receptors or receptor-class differences in synaptic localization might impact the relative D1/D2-stimulation (Durstewitz and Seamans 2008). Thereby, neurophysiological effects may be mediated, which underlie the dual state. For instance, D4- and D1-receptors are expressed on pyramidal cells and GABAergic interneurons, where D4-receptors have been shown to attenuate both NMDA-mediated synaptic responses of pyramidal neurons and their inhibition via GABAergic interneurons (Seamans, Gorelova et al. 2001; Wang et al. 2003). Generally, D1- and D2-like (including D4) receptor function is closely interwoven in prefrontal systems, with partly antagonistic effects of both receptor types. It has, for example, been shown that D2/D4-receptor stimulation can truncate D1-mediated excitation in time, thereby acting as an inhibitory modulator of D1 action (Seamans, Gorelova et al. 2001). Moreover, relative activity of the 2 receptor classes seems to partly depend on the background dopaminergic tone and the strength of phasic (task-related) stimulation (Seamans and Yang 2004).

Our findings of an opposite effect of D4-receptor sensitivity (7R- vs. no 7R-carriers) in *COMT* Val/Met versus Val/Val carriers (Fig. 2) are in line with the above-mentioned physiological principles: Under conditions of intermediate DA availability (Val/Met), higher values of the NGA (and lower Go-RT and Go-RT SD) were observed in the *DRD4* no 7R group, that is, subjects exhibiting relatively increased D4-receptor sensitivity. In this case, relatively increased D4-receptor functioning may provide optimal antagonism to (excitatory) D1 activity, resulting in optimal SNR and PFC processing. Under conditions of reduced DA availability (*COMT* Val/Val), however, higher NGA values were observed in subjects with a putatively reduced D4-receptor function (*DRD4* 7R). In terms of the above-mentioned model, this is also plausible as—under conditions of low DA availability and, thus, low D1-receptor stimulation—inhibitory effects of D4-receptor activation might be less beneficial for PFC functioning. In turn, low SNR present at low DA levels (Val/Val) (Winterer et al. 2006) may be enhanced when D4-receptors act less inhibitory (7R genotype) on interneuronal networks, thereby enhancing signal integration and the neural processing underlying the NGA (Fig. 2*a*). Thus, hypodopaminergic states (Val/Val) may be compensated by decreased D4 function, enhancing neural tuning in PFC circuits. Note that a significant impact of *DRD4* genotype on NGA values was observed in both *COMT* Val/Val and Val/Met, but not in Met/Met carriers. Therefore, high SNR in hyperdopaminergic states (Met/Met) may attenuate the impact of *DRD4* genotype on neurophysiological and behavioral measures of prefrontal response control. These findings and the dual-state theory are partly supported by a recent study of an interaction of *COMT* genotype and sulpiride, a selective DA D2-receptor blocker, on neural and behavioral correlates of error processing (Mueller et al. 2011). Here, *COMT* Val+ carriers (combined group of Val/Val and Val/Met) showed a relative decrease in error-related negativity (generated in the anterior midcingulate cortex) under sulpiride compared with Val+ under a placebo, whereas in Met/Met carriers, sulpiride had no significant impact. Behaviorally, these neural findings were mirrored by similar *COMT* × medication effects on posterror RT slowing (PES). Using a different EEG task and investigating gene–gene interactions, we detected similar neural (and behavioral) effects when comparing Val/Met carriers in our study with Val+ carriers investigated by Mueller et al. (2011). In these *COMT* groups, a decrease in (inhibitory) DA receptor function is accompanied with a decrease in neural activation (and task performance). However, our study separately investigating Val/Met carriers might allow for a more refined analysis and interpretation: Intermediate DA levels (Val/Met) are usually thought to be beneficial for neuronal tuning mechanisms underlying the inverted U-relationship of D1-receptor stimulation and stable neural representations as required, for example, for working memory (Seamans and Yang 2004; Vijayraghavan et al. 2007). According to the dual-state theory, stability of neural representations associated with a D1-dominated state (at intermediate DA levels) might be accompanied with the risk of decreased flexibility when D2-like receptor function is reduced. Here, *DRD4* genotype may play an important role, as D2-like receptors have been implicated in flexible neural integration of new information (Durstewitz and Seamans 2008). Reduced NGA (and increased Go-RT and Go-RT SD) may, thus, be a result of dysbalanced ratio of D1/D2-like receptor stimulation present in *COMT* Val/Met carriers with decreased D4-sensitivity (*DRD4* 7R). According to such an interpretation, reduced neural flexibility would result in less efficient transitions from neural Go to NoGo representations, thereby limiting performance, that is, increasing Go-RT and Go-RT SD (for example, due to a compensation strategy to prevent commission errors).

Importantly, cognitive response control is a multifactorial process that requires both cognitive stability and cognitive flexibility to be dynamically balanced (Cools and D'Esposito 2011). Thus, a balance of D1 and D2 states may be required for optimal neural processing and behavioral performance during cognitive response inhibition. Our results suggest that in Val/Met carriers (increased excitatory D1-stimulation) with a genotype-dependent decrease in inhibitory DA-receptor function (D2-like receptor family), this balance might be critically disturbed resulting in decreased NGA and, possibly to prevent commission errors during NoGo trials, an increase in response time and its variability (not significant after Bonferroni correction).

Neuroanatomically, the influence of *DRD4* genotype on the NGA within *COMT* Val/Met carriers was localized to the right premotor and supplementary motor area (Brodmann area 6), which has been shown to be activated during response inhibition (Aron et al. 2007; Congdon et al. 2009). Moreover, these regions are strongly connected to the subthalamic nucleus projecting to the globus pallidus, which may inhibit the cortico-subthalamic program underlying the Go response (Aron and Poldrack 2006). The neural regulation of the cortico-subthalamic loop and fronto-striatal-thalamic networks mediating response inhibition behavior (Stevens et al. 2007) has been suggested to be modulated by genotype-

dependent DAT (Dresler et al. 2010; Cummins et al. 2011) as well as by COMT function (Congdon et al. 2009). Here, our results extend previous findings by showing that on the level of the right premotor and supplementary motor area, the epistatic interaction of *COMT* and *DRD4* significantly impacts neural functioning of cognitive response control. However, other prefrontal structures, such as the inferior and middle frontal gyri, have been implicated to play an important role in response inhibition (Chikazoe 2010). Future imaging genetic studies should address the issue of a possibly differential impact of (dopaminergic) genotypes on neural processing within different brain regions involved in response inhibition.

Previous studies investigating an interaction of *DRD4* and *COMT* genotype on behavior and neural activation in healthy subjects were conducted using reward (Marco-Pallares et al. 2009; Camara et al. 2010) and performance monitoring paradigms (Kramer et al. 2007). These studies found no epistatic effect of *DRD4* × *COMT* (*DRD4*-521 C/T and *COMT* Val158Met), possibly due to small sample sizes and the systematic exclusion of heterozygous *COMT* Val/Met carriers.

While the present study aimed to investigate a genetic impact on the NGA as a relative measure (Go–NoGo) of response control processes linked to the ACC, *COMT* × *DRD4* epistasis showed a similar but less pronounced effect on the level of position- and condition-dependent P300 amplitudes. Again, this epistatic interaction was not significantly modulated by ADHD diagnosis. Moreover, while the negative correlation of NGA and performance measures was weak ($r = -0.12$), P300 amplitudes underlying the NGA effect showed highly significant negative correlations with Go-RT and Go-RT SD with values between −0.27 and −0.52, substantiating the relevance of these neural measures for inhibitory control. In line with a meta-analysis on the Go and NoGo P300 in adult ADHD (Szuromi et al. 2011), ADHD patients showed reduced P300 amplitudes compared with controls, particularly in the NoGo condition. The significant interaction of diagnosis, condition, and electrode position (Fig. 3*a*,*b*) indicated altered (topographical) P300 characteristics in a patient group with putative problems in cognitive control and response inhibition. Taken together, the present findings suggest COMT × DRD4 epistasis to exert an impact on prefrontal processing independent of ADHD diagnosis, whereas in ADHD, additional neural alterations might be involved affecting P300 amplitudes and Go-NoGo task performance.

Some limitations of our study have to be considered. (1) Although the sample size is large for imaging genetic studies, it is relatively small regarding the multitude of (epi) genetic factors, which may also impact prefrontal processing. (2) The influence of other neurotransmitter systems modulating prefrontal processing such as glutamate, GABA, and serotonin was not investigated. (3) In ADHD patients, the criteria for HWE regarding *COMT* Val158Met were not met. This disequilibrium ($P = 0.045$) was due to a slightly increased number of *COMT* homozygotes in ADHD patients, which, however, should not affect the *COMT* × *DRD4* epistasis (in *COMT* heterozygotes). (4) As common in this clinical population, 49.2% of the ADHD patients had a current comorbid disorder (see Supplementary Material for further details). While our sample of ADHD patients may, therefore, be representative, we cannot exclude the possibility that features of the comorbid disorders introduced some error variance in our data. However, introducing the presence of a comorbid disorder as a fixed factor had no significant impact on gene main or epistatic effects. (5) ADHD patients showed no statistical difference in NGA compared with healthy controls, which is in contrast to previous findings (Fallgatter et al. 2005). However, after excluding ADHD patients with any comorbid disorder, the remaining patients ($n = 92$) showed the expected decreased NGA compared with healthy controls ($t_{204} = -2.09$, $P = 0.038$, $d = 0.29$). Even the reduced sample of controls and ADHD patients ($n = 206$) showed the epistatic *COMT* × *DRD4* interactions on the NGA ($P = 0.016$), Go-RT ($P = 0.028$), and a relatively increased epistatic effect on Go-RT SD ($P = 0.006$). (6) Due to the limited number of electrodes used in this study (21 scalp electrodes), the validity of our sLORETA findings may be reduced, and therefore comparison of (smaller) genotype groups might lack sufficient statistical power to additionally show genotype effects in inferior frontal gyrus and other frontal regions contributing to variance of the NGA. Therefore, our sLORETA results should be considered exploratory and require further validation.

Considering the interdependency of DA availability with specific DA receptor functions on SNR and network dynamics (stable vs. flexible) might help to disentangle neural mechanisms of prefrontal functioning. For instance, attention and executive function deficits in ADHD and schizophrenic spectrum disorders, which may partly emerge from imbalanced integration of DA signals via D1- and D2-like receptors (Durstewitz and Seamans 2008), might also have to be viewed in the light of (*COMT*-dependent) DA availability. Specifically, intermediate prefrontal DA levels (*COMT* Val/Met genotype) might represent a dopaminergic state in which prefrontal processing is increasingly vulnerable to decreased D4-receptor sensitivity (*DRD4* 7R-allele), which profoundly impacts prefrontal functioning underlying cognitive response control. Furthermore, our findings may provide important hypotheses for future imaging genetic studies investigating the dopaminergic regulation of prefrontal function as well as genetic association studies of ADHD and schizophrenia considering genetic epistasis.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

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Study #1: Online supplementary data and information

Subjects and Methods

Comorbidities within the ADHD patient group:

Of 181 adult ADHD patients included in the study 49.2% also had a current psychiatric comorbid axis I disorder as evaluated with the Structured Clinical Interview for DSM-IV (SCID-I) (Wittchen H et al., 1997):

22 patients were also diagnosed with substance misuse/dependency (alcohol abuse [F10.1; n=2] or dependency [F10.2; n=3]; cannabinoid abuse [F12.1; n=3] or dependency [F12.2; n=13]; stimulant dependency [F15.2; n=1]),

37 with mood disorders (bipolar affective disorders [F31.0, F31.8; n=6], depressive episodes [F32.1, F32.8; n=3], recurrent depressive episodes [F33.0, F33.1; n=8], cyclothymia [F34.0; n=3], dysthymia [F34.1; n=9], and other recurrent mood disorders [F38.1; n=8]),

30 with neurotic, stress-related and somatoform disorders (agoraphobia [F40.0; n=1], social phobias [F40.1; n=7], specific phobias [F40.2; n=7]; panic disorder [F41.0, F40.01; n=6]; generalized anxiety disorder [F41.1; n=3], obsessive compulsive disorder [F42.0; n=1], post-traumatic stress disorder [F43.1; n=3], bulimia nervosa [F50.2; n=1] and unspecified eating disorders [F50.9; n=1]).

Artifact criterions of electrophysiological data:

Three additional electrodes were attached at the outer canthi of both eyes and below the right eye for registration of eye movements. Data were corrected for ocular artifacts using the algorithm by Gratton and Coles (Gratton G and MGH Coles, 1989) and further epochs/segments were excluded if they contained any additional or remaining motor or technical artifact. In this regard, epochs containing artifacts (exclusion criteria: amplitudes > 70 µV or < -70 µV in any of the EEG-channels within -100 ms to +700 ms relative to stimulus presentation [amplitude criterion]; or voltage steps > 70 µV from one sampling point to the next [gradient criterion]) were rejected. Only artifact-free epochs with correct behavioral responses were segmented and individually averaged to Go and NoGo event-related potentials (ERPs). The minimum number of trials averaged per participant and condition was n = 20.

Post-hoc tests

Post-hoc tests of *COMT* and *DRD4* epistasis on Go-RT.

One-way ANOVAs (dependent variable: Go-RT; between-subject factor *COMT* (number of Met-alleles)) and subsequent post-hoc tests were applied separately for the *DRD4* genotype groups ("No 7R", "7R"):

No 7R group: $F_{2, 174} = 1.76$; $p = .18$

7 R group: $F_{2, 115} = 2.94$; $p = .06$

Significant results of post-hoc tests:

7R group: Go-RT (Val/Met-Met/Met): $t_{75} = 2.43$; $p = .02$

Comparisons (t-tests of independent samples) of Go-RT between *DRD4* genotype groups

(No 7R-7R); separately for *COMT* genotype subgroups:

COMT Val/Val: $t_{80} = 1.02$; $p = .31$

COMT Val/Met: $t_{128} = -2.16$; $p = .03$

COMT Met/Met: $t_{81} = 1.74$; $p = .09$

Post-hoc tests of *COMT* and *DRD4* epistasis on Go-RT SD.

One-way ANOVAs (dependent variable: Go-RT SD; between-subject factor *COMT* (number of Met-alleles)) and subsequent *post-hoc* tests were applied separately for the *DRD4* genotype groups ("No 7R", "7R"):

No 7R group: $F_{2, 174} = 4.46$; $p = .01$

7 R group: $F_{2, 115} = 1.90$; $p = .16$

Significant results of post-hoc tests:

No 7R group: Val/Val-Val/Met: $t_{120} = 2.61$; $p = .01$

Val/Met-Met/Met: $t_{134} = -2.47$; $p = .02$

7R group: Val/Met-Met/Met: $t_{75} = 1.73$; $p = .09$

Comparisons (t-tests of independent samples) of Go-RT SD between *DRD4* genotype groups (No 7R-7R); separately for *COMT* genotype subgroups:

COMT Val/Val: $t_{80} = 96$; $p = .34$

COMT Val/Met: t_{128} = -3.30; p = .001

COMT Met/Met: t_{81} = 1.12; p = .27

Post-hoc tests of *COMT* and *DRD4* epistasis on NGA.

One-way ANOVAs (dependent variable: NGA; between-subject factor *COMT* (number of Met-alleles)) and subsequent post-hoc tests were applied separately for the *DRD4* genotype groups ("No 7R", "7R"):

No 7R group: $F_{2, 174}$ = 3.96; p = .02

7 R group: $F_{2, 115}$ = 3.58; p = .03

Significant results of post-hoc tests:

No 7R group: Val/Val-Val/Met: t_{120} = -2.72; p = .007

Val/Met-Met/Met: t_{134} = 2.05; p = .04

7R group: Val/Val-Val/Met: t_{88} = 2.57; p = .01

Val/Met-Met/Met: t_{75} = -1.68; p = .09

Comparisons (t-tests of independent samples) of NGA between *DRD4* genotype groups (No 7R-7R); separately for *COMT* genotype subgroups:

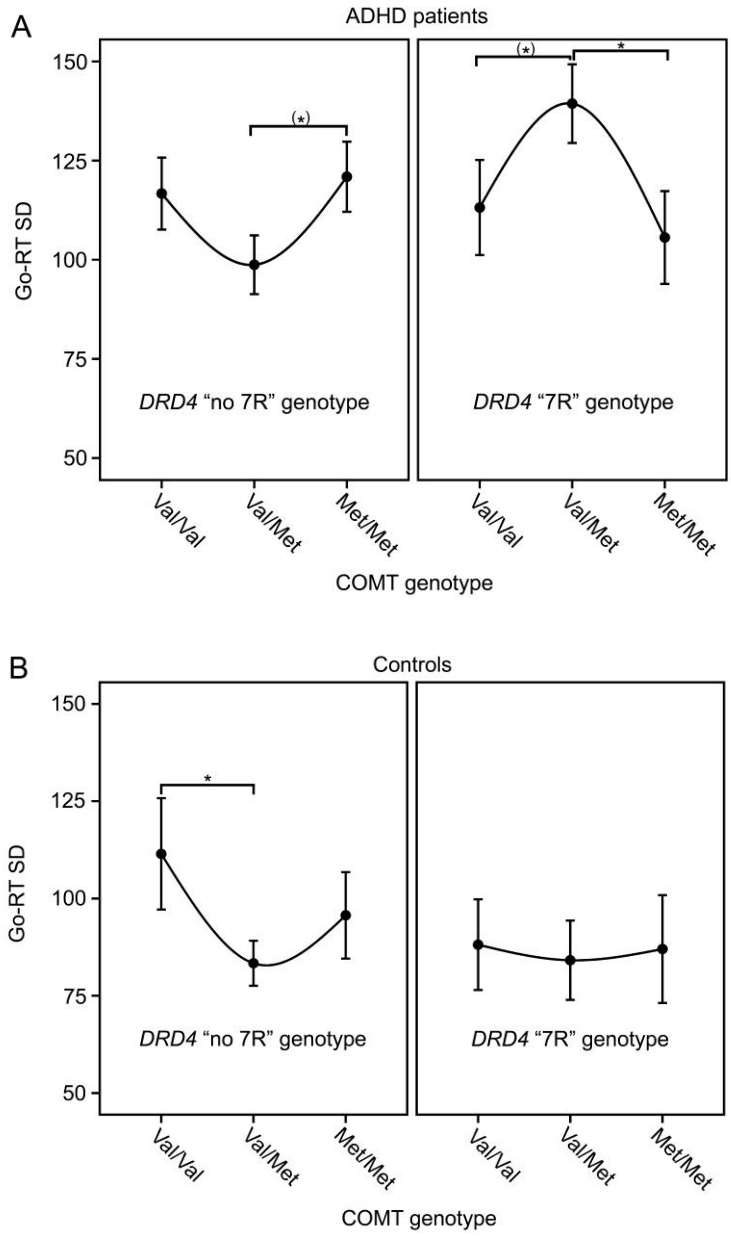
COMT Val/Val: t_{80} = -2.26; p = .03

COMT Val/Met: t_{128} = 3.20; p = .002

COMT Met/Met: t_{81} = -.90; p = .37

Comparison of diagnostic groups

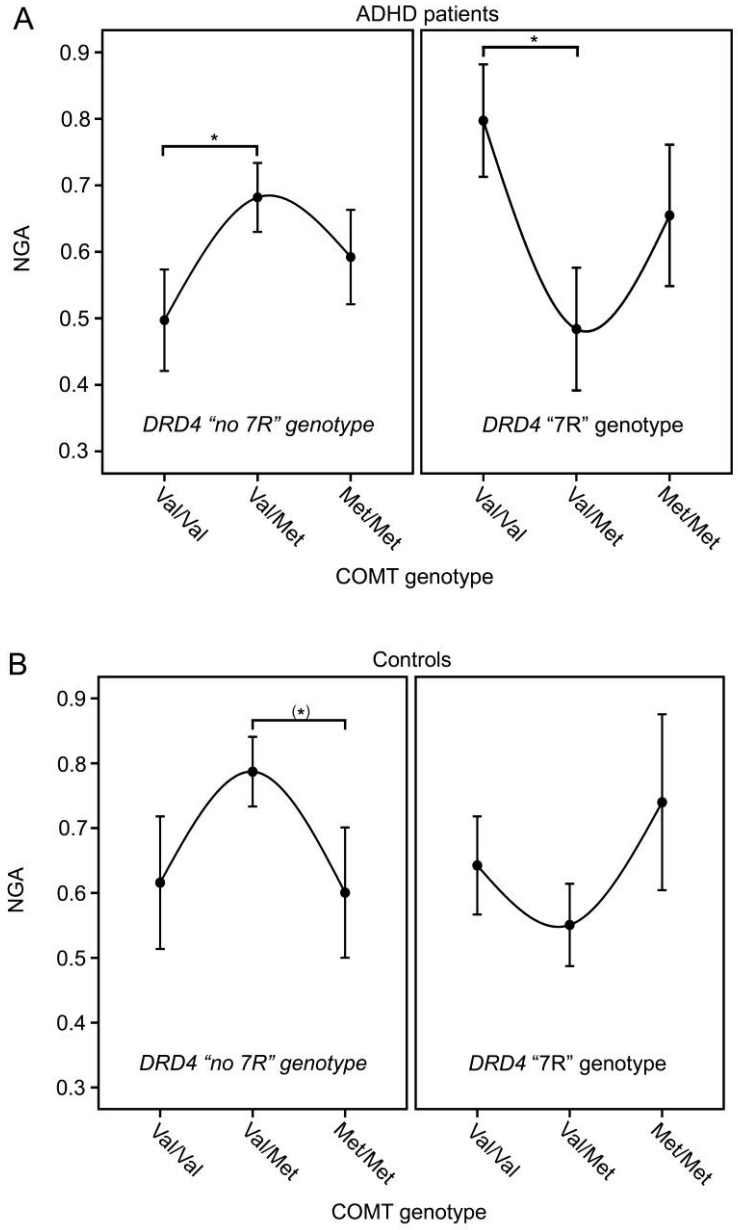
Diagnosis did not significantly impact *COMT*×*DRD4* epistasis on Go-RT SD (p > .3). Exploratory analyses revealed that the epistatic interaction described (inverted) U-shapes in ADHD patients as well as controls (see Supplementary Figure 1 A, B), however, controls with *COMT* Val/Met and *DRD4* 7R genotype do not display increased Go-RT SD compared to corresponding ADHD patients (t_{47} = 3.47; p = .001).



Supplementary Figure 1: (A) Go-RT SD follows an (inverted) U-curve with increasing number of COMT Met-alleles in ADHD patients with *DRD4* "no 7R" and "7R" genotype, respectively. (B) Healthy controls with "*DRD4* no 7R" genotype also exhibit a U-relationship of Go-RT SD over *COMT* subgroups, however, in 7R carriers the inverted U-relationship is not present. Note that in the ANOVA (diagnosis x *COMT*×*DRD4*) diagnosis did not explain further variance regarding the *COMT*×*DRD4* interaction. Exploratory (*post hoc*) analyses show that ADHD patients with *COMT* Val/Met and *DRD4* 7R genotype have a higher Go-RT SD compared to corresponding healthy con-

trols (t_{47} = 3.47; p = .001). Error bars indicate standard error of the mean (SEM). Asterisks and asterisks in brackets indicate significant differences at a significance level of p < .05 and p < .10, respectively.

Similarly, *COMT*×*DRD4* epistasis on NGA was not significantly impacted by diagnosis (p > .3), thus, (inverted) U-relationships are similar and *post hoc* comparisons revealed no diagnosis-related differences in NGA (see Supplementary Figure 2 A, B).



Supplementary Figure 2: (A) NGA describes an (inverted) U-curve over *COMT* subgroups in ADHD patients with *DRD4* "no 7R" and "7R" genotype, respectively. (b)

Healthy controls with "*DRD4* no 7R" genotype also exhibit (inverted) U-relationships of NGA over *COMT* subgroups with *DRD4* "no 7R" and "7R" genotype, respectively. Error bars indicate SEM.

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Cumulative dissertation of Dipl.-Biol. Sebastian Heinzel:
Declaration of own and co-author contributions

Manuscript:

"Aging-related cortical reorganization of verbal fluency processing: an fNIRS study"

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Planning of fNIRS measurements: SH, AJF, KH, FM, ACE

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Database of behavioral and demographic information: SH, RK, AA

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fNIRS and behavioral/demographic data analysis and interpretation: SH

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Aging-related cortical reorganization of verbal fluency processing: a functional near-infrared spectroscopy study

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Abstract

While progressive neurocognitive impairments are associated with aging and Alzheimer's disease (AD), cortical reorganization might delay difficulties in effortful word retrieval, which represent one of the earliest cognitive signs of AD. Using functional near-infrared spectroscopy (fNIRS), we investigated cortical hemodynamic responses elicited by phonological and semantic verbal fluency in non-demented, healthy subjects (n = 325; age: 51–82 years). Age predicted bilaterally reduced inferior frontal junction (IFJ) and increased middle frontal and supramarginal gyri activity in both task conditions using multiple regressions. Compared with age the years of education as well as sex (IFJ activation in females > males) partly predicted opposite effects on activation, while task performance was not significant predictor. All predictors showed small effect sizes. IFJ activation was more pronounced during phonological compared with semantic fluency, and higher in the left hemisphere. Age only marginally predicted relative lateralization. Middle frontal and supramarginal gyri activity may compensate for an aging-related decrease in IFJ recruitment during verbal fluency. Longitudinal observations will further investigate these neural changes regarding an early AD prediction, while individuals are still cognitively healthy.

Keywords: Aging; Alzheimer prediction; Sex; Functional near-infrared spectroscopy (fNIRS); Verbal fluency test (VFT); TREND study (Tuebinger evaluation of Risk factors for Early detection of NeuroDegeneration)

1. Introduction

The first cognitive symptoms in neurodegenerative conditions such as Alzheimer's disease (AD) are often consequences of altered metabolic, vascular, neuronal, and neural network processes preceding diagnosis by many years or even decades (Jack et al., 2010; Perrin et al., 2009). These changes may evoke compensatory and reorganizational processes to overcome impairment of cellular and neural system functioning caused by aging and neurodegeneration (Burke and Barnes, 2006; Cabeza, 2002; Davis et al., 2008; Liang et al., 2011). For instance, additional recruitment of (contralateral) neural areas, not typically activated during a particular task in younger subjects, might represent neural reorganization of function in older individuals. Thereby, task performance may not be different from younger individuals and the onset of cognitive and behavioral symptoms of neurodegeneration may be temporarily delayed. Such functional reorganization might emerge from inefficient re-

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cruitment and processing of specialized neuronal ensembles during aging as reflected by decreases in activation size and magnitude within core brain regions related to specific cognitive or executive functions (Beason-Held et al., 2008; Eyler et al., 2011). Thus, both relative decreases and (compensatory) increases in functional brain activation may represent consequences of aging-related alterations occurring at multiple organizational levels of the brain.

Despite efforts to incorporate biomarkers into the diagnosis of AD (Sperling and Johnson, 2012), a reliable diagnosis is currently only possible when the neurodegenerative process has far progressed. Thus, there is an urgent need for the identification of risk factors and prodromal markers of the disease to allow an earlier diagnosis. While high chronologic age is an undisputed risk factor for AD, current evidence suggests that smoking, major depression, midlife obesity, and cognitive inactivity, as well as vascular and metabolic dysfunction presented in disorders such as diabetes mellitus, dyslipidemia, and midlife hypertension represent additional major AD risk factors (Barnes and Yaffe, 2011; Murray et al., 2011). Although all these factors are known to be associated with an increased risk for AD, their sole existence does not contribute to an earlier diagnosis of AD. However, changes in cognitive neural processing might serve an early individual diagnosis of mild cognitive impairment (MCI) and subsequent AD before the onset of cognitive deficits.

The **T**übinger evaluation of **R**isk factors for **E**arly detection of **N**euro**D**egeneration study (TREND) is a prospective, longitudinal study that aims at identifying markers predicting AD and Parkinson's disease, respectively, by assessing multiple neural, vascular, neuropsychological, and motor measures, as well as genetic information and blood-based biomarkers in 1200 neurodegeneratively healthy, non-demented elderly subjects every 2 years until autopsy (Berg, 2012; Hobert et al., 2011). Thereby, participants within an age range (50–80 years old) of high incidence for neurodegenerative diseases are followed up and data underlying and predicting an eventual transition from a healthy condition to mild cognitive impairment or AD (or Parkinson's disease, respectively) can be collected within the longitudinal TREND study.

Among numerous other assessments, neural correlates of cognitive function are being investigated using functional near-infrared spectroscopy (fNIRS) measurements of cortical hemodynamic responses elicited by means of a verbal fluency test (VFT). The VFT is a demanding task, challenging cognitive function during effortful retrieval and verbal articulation of words matching phonological or semantic criteria, which also requires several executive functions (Henry and Crawford, 2004; van Beilen et al., 2004). Difficulties in effortful word retrieval represent one of the earliest signs of AD (Henry et al., 2004).

Because of its relatively low susceptibility to motion artifacts (Dieler et al., 2012; Schecklmann et al., 2010), fNIRS has become a promising technique for investigating neural correlates of cognitive function underlying the VFT. Phonological and semantic verbal fluency have been shown to reliably elicit bilateral functional hemodynamic responses within inferior frontal gyri (IFG), middle frontal gyri (MFG), and fronto-temporal regions measured by using multichannel fNIRS systems (Dresler et al., 2012; Ehlis et al., 2007; Herrmann et al., 2006; Kakimoto et al., 2009; Reif et al., 2011; Richter et al., 2007; Schecklmann et al., 2007, 2008, 2010; Suto et al., 2004). Pronounced left compared with right hemispheric activation as well as higher fronto-temporal activation for the phonologic compared with the semantic condition has been shown in healthy, young subjects (Ehlis et al., 2007; Schecklmann et al., 2008).

Studies using functional magnetic resonance imaging (fMRI), positron emission tomography or single-photon emission computed tomography found activation within the left IFG and MFG as well as activation within anterior cingulate cortex, putamen, thalamus, and cerebellum, which are too distant from the skull to be detected using fNIRS (Abrahams et al., 2003; Audenaert et al., 2000; Birn et al., 2010; Meinzer et al., 2009; Ravnkilde et al., 2002). Also, within the left IFG phonologic fluency yielded stronger activation compared with semantic fluency conditions (Birn et al., 2010; Heim et al., 2008; Meinzer et al., 2009).

So far, findings regarding sex differences in VFT-related activation (Gauthier et al., 2009; Herrmann et al., 2006; Kameyama et al., 2004; Richter et al., 2007; Weiss et al., 2003) as well as regarding the impact of age on neural correlates of verbal fluency (Herrmann et al., 2006; Kameyama et al., 2004; Meinzer et al., 2009) have been inconsistent. However, compared with healthy controls, AD patients have been shown to exhibit reduced lateral frontal cortex activation during phonological and semantic VFT conditions (Herrmann et al., 2008; Richter et al., 2007). Also, in AD patients, metabolism within the left inferior frontal junction (IFJ) area and left prefrontal cortex blood flow changes have been shown to be negatively correlated with semantic verbal fluency performance (Kitabayashi et al., 2001; Schroeter et al., 2012).

Neural signatures of prodromal stages of MCI or AD and aging-related changes in phonologic and semantic verbal fluency processing have not yet been studied in large-scale samples of non-demented elderly subjects.

We, therefore, investigated the impact and effect sizes of age and sex on cortical processing of verbal fluency and aging-related reorganization of neural processing using fNIRS data in an exceptionally large sample (n = 325). Longitudinal investigations of these correlates of neural processing, also in combination with multiple other risk factors and biomarkers, may help to specify antecedent markers of neurodegeneration, especially when considering longitudinal data profiles of subjects developing MCI or AD.

2. Methods

2.1. Participants

A total 379 subjects were assessed in the TREND study between March and April 2011. Fifty subjects were excluded from the present fNIRS analyses because of regular intake of acetylsalicylic acid (e.g., aspirin), which has been shown to modulate neurovascular coupling (Gordon et al., 2011). Additionally, 2 subjects were excluded because of technical problems and 2 subjects because they were not German native speakers, resulting in 325 healthy, non-demented, elderly subjects (203 female, 122 male) included in the present analyses. This sample comprised 271 right-handed, 18 left-handed, and 8 ambidextrous (former left-handers) subjects (self-assessment of handedness was not available for 28 subjects). One important scope of the TREND study is to inform participants about potential signs of a dementia (or Parkinson's disease) diagnosis. Therefore, for participants showing overly poor performance, e.g., in neuropsychological tests, for instance, the Mini Mental State Examination as a common screening instrument was analyzed immediately. Thus, in the present study dementia could be excluded as indicated by a Mini Mental State Examination score > 23.

Mean age was 64.6 $\pm$ 7.3 years (range: 51–82 years). Compared with male participants (67.0 $\pm$ 7.4 years; range: 52–82 years) female participants (63.2 $\pm$ 6.9 years; range: 51–79 years) were significantly younger ($t(323) = -4.62$; $p < 0.001$). Therefore, all sex-related analyses were repeated with a reduced sample matched for age ($n = 272$; $t(270) = -0.029$; $p = 0.98$). Total years of formal education for the whole group were 14.3 $\pm$ 2.9 years, for females 13.8 $\pm$ 2.9 years, and for males 15.1 $\pm$ 2.7 years. Thus, there was a significant sex difference ($t(320) = -4.02$; $p < 0.001$; values from 3 subjects were not available) in the education time.

For a detailed outline of the TREND study, further inclusion and exclusion criteria, and baseline assessments, see Berg (2012). Importantly also for this sub-study, all participants were pre-screened via telephone interview before study inclusion in 2009/2010, and were excluded if they reported a history of psychiatric diseases (other than unipolar major depression), dementia, epilepsy, stroke, multiple sclerosis, encephalitis, and malignancies, and intake of antipsychotics and other drugs that may promote Parkinsonian symptoms. The study was approved by the ethical committee of the Medical Faculty of the University of Tuebingen (Nr. 90/2009BO2). All procedures were in accordance with the Declaration of Helsinki in its latest version, and all subjects gave written informed consent.

2.2. Verbal Fluency Test (VFT)

The VFT comprised three different trial conditions in which participants had to name as many words as possible within 30 seconds meeting the instructed category criteria: (1) phonologic version: producing nouns beginning with a given letter (A, F, and M); (2) semantic version: producing words from a certain category (professions, fruits, and flowers); and (3) control task: reciting of weekdays in a consecutive manner. Here, the pace of word generation was adjusted by oral instruction, to slow down or to accelerate the pace, yielding a similar number of generated words compared with the other (active) task conditions. Generally, word repetitions and proper names were not allowed. Each trial was followed by a resting phase of 30 seconds. The participants were instructed to stay focused on the experimental task regardless of the individually experienced performance (errors) and to verbally articulate as many words as possible meeting the task criteria. In the resting phase, participants were asked to relax and discontinue mental engagement regarding previous and consecutive word generation or task demands. Moreover, participants were instructed to avoid movements and to close their eyes during the entire measurement.

The total measurement duration was 550 seconds. The VFT performance, i.e., the mean number of correct words of all three 30-second trial blocks of the phonologic, semantic, and control condition, respectively, was assessed. See Fig. 1 for an illustration of the VFT protocol.

2.3. fNIRS

Neural activity was inferred by the relative changes in concentration of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb), respectively, which were recorded with an

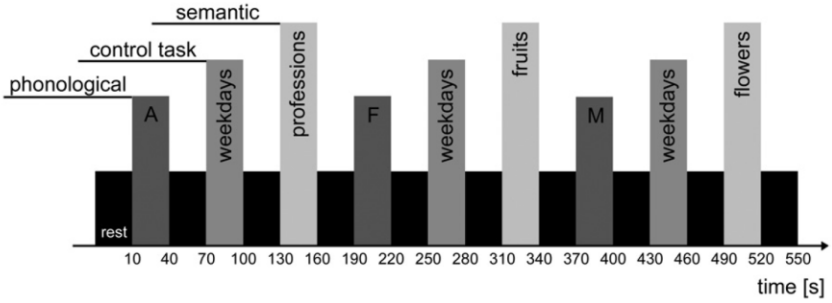

Fig. 1. Illustration of the experimental verbal fluency test (VFT) protocol.

fNIRS continuous wave two system (ETG-4000 Optical Topography System, Hitachi Medical Corporation, Tokyo, Japan) at a sampling rate of 10 Hz and an inter-optode distance of 3 cm. We used 2 probe sets each consisting of 8 semiconductor laser diodes (wavelengths: 695 ± 20 and 830 ± 20 nm) and 7 detectors in a 3 × 5 probe array (area of 12 × 6 cm) resulting in 22 recording channels for each hemisphere.

The probe sets were bilaterally adjusted according to the international 10–20 system for electrode placement. Specifically, for the left-sided probe set, the detector located equidistant between channel 1 and 2 was positioned on the marker T3, whereas for the right-sided probe set the corresponding detector was positioned on T4. Furthermore, the most caudal row of optodes was adjusted on a horizontal line of Fpz-T3 and Fpz-T4, respectively. This spatial information was used to calculate projections of the superficial optode and channel positions onto a standard brain using functions of the National Food Research Institute in Japan (www.jichi.ac.jp/brainlab/tools.html); (Singh et al., 2005; Tsuzuki et al., 2007). Thereby, Montreal Neurological Institute coordinates, errors of estimation (standard deviations) given by the radius of the circle indicating a projected channel (ranging between 4 and 10 mm; see Fig. 2), as well as probabilistic anatomic labeling of each fNIRS recording channel were determined. According to these probabilistic values of automated anatomical labeling (AAL) of brain regions (Tzourio-Mazoyer et al., 2002), fNIRS channels recorded functional hemodynamics within the (pre)frontal, temporal, and parietal cortex. Specifically, functional hemodynamic responses within IFG (including orbital, opercular, and triangular areas), MFG (with orbital areas), and superior frontal gyrus (dorsolateral), precentral and postcentral gyrus, Rolandic operculum, as well as inferior parietal gyrus, supramarginal gyrus, angular gyrus, middle temporal gyrus (including the temporal pole), and superior temporal gyrus were measured (only AAL regions with a probability > 10% are listed). In the following, fNIRS channels will only be anatomically labeled after the AAL region of the highest probability. Because for channel 3 IFG and temporal pole reached comparable labeling probabilities, channel 3 will be referred to as IFJ. See Fig. 2 for channel positions and numbering.

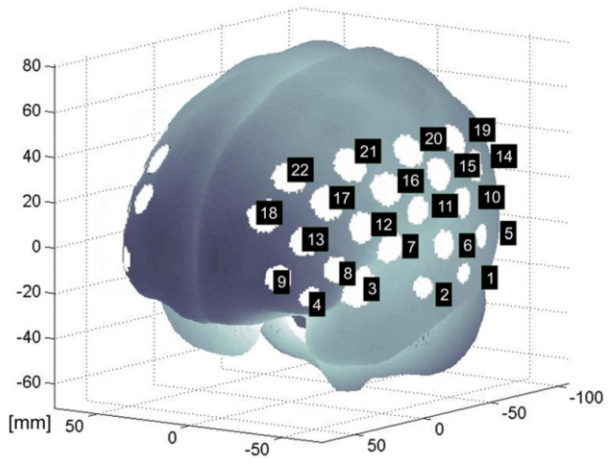

Fig. 2. Brain model with projected functional near-infrared spectroscopy (fNIRS) channels and numerical labeling.

A moving average filter with a time window of 5 seconds and a bandpass filter (lowpass: 1/2 Hz; highpass: 1/128 Hz) were applied to remove slow drifts and high frequency fluctuations in the fNIRS O_2Hb and HHb time series data. Functional hemodynamics measured using fNIRS might be affected by systemic biosignals, such as extra- and intracranial changes in blood pressure and blood flow due to arousal (Minati et al., 2011; Takahashi et al., 2011). In order to correct the hemodynamic data for these potential confounders, the mean sampling point value of a probe set (22 channels) was subtracted from the data of each sampling point of each single channel of that probe set (common average reference; CAR). While this procedure may allow for a more specific identification of regions exhibiting functional hemodynamic responses reflecting neural processing, signal changes in each channel are only relative to the mean probe-set response and not absolute, i.e., relative decreases can indicate small signal increases in comparison with the mean hemodynamic response of the whole probe set. Without this mean correction procedure no signal decreases were present. Instead, even the channel with the smallest response amplitude (characterized by a signal decrease after the CAR procedure) still showed a significant signal increase in the raw data. The general shape of the activation topography did not notably differ between the two analysis strategies. For event-related averages of O_2Hb data without mean correction see the Supplementary data.

Neuronal activation is generally accompanied with an increase in O_2Hb and decrease in HHb fNIRS signals, respectively. O_2Hb has been suggested to have a better signal-to-noise ratio and to represent the most sensitive and reliable indicator of changes in regional cerebral blood flow, while HHb sometimes shows paradoxical signal changes (Hoshi, 2007; Hoshi et al., 2001; Plichta et al., 2007; Yamamoto and Kato, 2002). However, HHb has been suggested to have a better regional specificity (Hirth et al., 1996). The O_2Hb signal was the primary dependent variable of the fNIRS data and analyses of HHb data are given in the Supplementary data.

After a baseline-correction of the fNIRS data (baseline from −2 seconds to trial onset) an event-related average was performed and mean hemodynamic response amplitudes (within 1 to 30 seconds) for each channel and each VFT condition were calculated.

2.4. Statistical analysis

As basis for statistical analyses mean amplitudes of the control task were subtracted from those of the experimental

conditions (Letter–Weekday; Category–Weekday) and experimental conditions were contrasted (Letter–Category). Lateralization of the resulting topography was investigated by contrasting mean amplitudes of corresponding bilateral channels. Significant increases and decreases were tested by means of a *t*-test (against 0) of the condition contrasts. In order to accommodate for the multiple testing situation, we applied false discovery rate (FDR) corrections (Benjamini and Hochberg, 1995; Singh and Dan, 2006) for all *t*-tests and Pearson correlations of 44 fNIRS channel recordings and a significance threshold of $p < 0.01$. Effect sizes *d* (Cohen, 1988) were calculated as the difference of the relative mean amplitudes of an experimental and the control condition divided by the mean standard deviation of the two conditions. Pearson correlations between the participants' age and relative mean amplitudes were calculated. Significant correlations (FDR corrected, $p < 0.01$) were then further analyzed using a multivariate approach. We calculated multiple regressions comprising the (dependent) criterion variable of the mean amplitudes of the channel-wise fNIRS signal (relative to CAR) of the phonological (Letter) and semantic (Category) condition, respectively, which were contrasted against the control condition (Weekday). The variables of age, sex (dummy coding: female = 0, male = 1), (condition-dependent) task performance and the years of education were included ("enter" method) as predictors of the regression model. Here, for significant findings effect sizes were indicated by the standardized regression coefficient (β). The percentage of variability of the criterion variable explained by the multiple regression model is indicated by R^2. For all multiple regressions, e.g., multicollinearity, normality, and homogeneity of variance met common multiple regression criteria. Because multiple regressions were focused on fNIRS channel activations previously shown to be significantly correlated with age (FDR-corrected, $p < 0.01$), no further correction for multiple testing was applied and predictors were considered significant at $p < 0.05$ and marginally significant at $p < 0.1$.

The fNIRS data analyses were performed using custom software written in MATLAB 7.9 (The MathWorks, Natick, MA, USA). Statistical analyses were performed using IBM/SPSS v19.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Behavioral data

In the phonologic (Letter) condition of the VFT participants pronounced 6.1 ± 2.0 (mean ± standard deviation) correct nouns, in the semantic (Category) condition 9.9 ± 2.0 correct words, and in the control task 9.3 ± 1.3 weekdays. Age was weakly negatively correlated with the semantic ($r = -0.13$; $p < 0.05$; $d = 0.26$) but not with phonologic VFT performance ($r = -0.09$; $p > 0.1$). Phonologic VFT performance ($r = 0.39$; $p < 0.001$; $d = 0.85$) was more strongly correlated with the number of years of education than semantic VFT performance ($r = 0.15$; $p < 0.01$; $d = 0.30$). Females pronounced more words than males in the phonologic ($t(323) = 2.3$; $p < 0.05$; $d = 0.26$; females > males: 0.5 ± .23 words) and in the semantic condition ($t(323) = 4.7$; $p < 0.001$; $d = 0.52$; females > males: 1.0 ± .22 words), but not in the control condition ($t(323) = 1.3$; $p > 0.1$). When correcting for sex differences in years of education these differences remained significant. However, when sex was matched for age, phonologic performance differences were no longer significant ($t(323) = 1.3$; $p > 0.1$), while semantic performance differences remained highly significant ($t(323) = 3.4$; $p < 0.001$; $d = 0.41$). Age was not significantly correlated with the years of education ($r = -0.11$; $p > 0.05$).

3.2. fNIRS data

The event-related averages of functional hemodynamic responses elicited by the different task conditions are shown (Fig. 3) for the fNIRS channel exhibiting the largest increase (channel 3, left) and the smallest O_2Hb increase (i.e., largest relative decrease; channel 14, left). While O_2Hb increased during performance of the VFT, the signal decreased during the resting phase. Statistical maps (*t*-tests) of channel-wise O_2Hb mean amplitude contrasts are shown in Fig. 4. Bilaterally, peak O_2Hb increases for the Letter–Weekday as well as the Category–Weekday contrast were detected in the IFG. The smallest O_2Hb signal increases were present within bilateral inferior parietal and middle frontal regions. For the Letter–Weekday contrast effect sizes (mean $d = 0.55 \pm 0.24$) ranged between $d = 1.02$ (channel 3) and $d = 0.09$ (channel 9) on the left side and between $d = 1.78$ (channel 3) and $d = 0.00$ (channel 12) on the right side ($d = 0.43 \pm 0.38$). For the Category–Weekday contrast smaller effect sizes on the left side ($d = 0.43 \pm 0.19$; range, 0.73 [channel 3] to 0.08 [channel 9]) as well as on

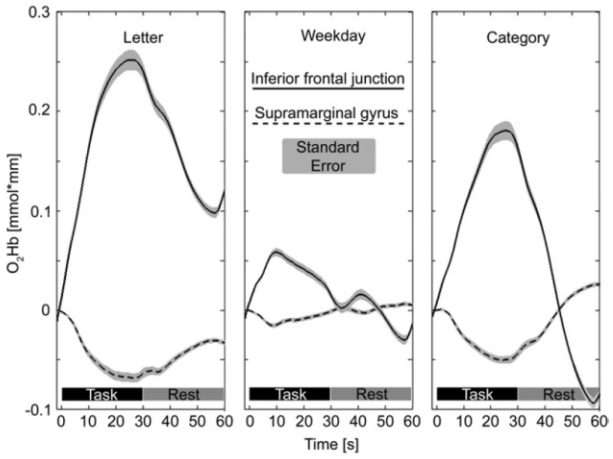

Fig. 3. Event-related average of functional hemodynamic responses elicited by phonologic (Letter), control (Weekday), and semantic (Category) conditions of the verbal fluency test (VFT).

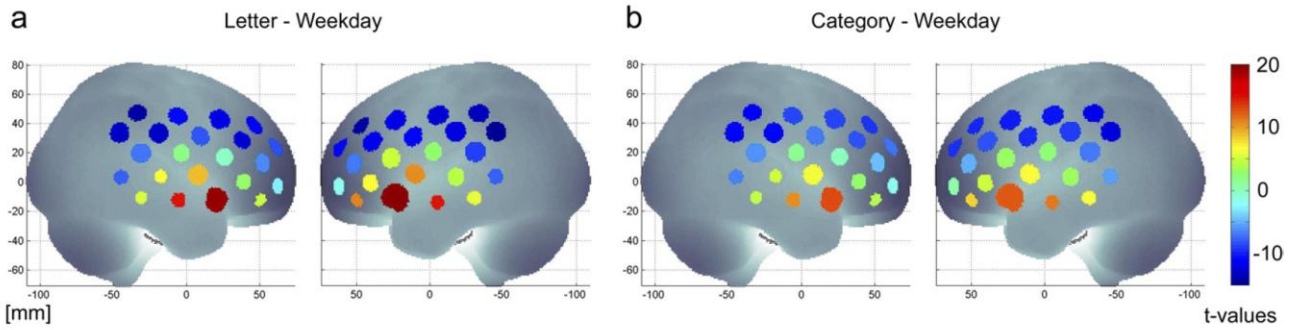

Fig. 4. Statistical *t*-value topography of functional changes in hemoglobin oxygenation. (a) Phonologic conditions contrasted with control conditions; (b) semantic conditions contrasted with control conditions.

the right side ($d = 0.37 \pm 0.20$; range, 0.75 [channel 3] to 0.02 [channel 12]) were observed, while the same channels showed maximal and minimal values, respectively. Contrasting the experimental conditions (Letter–Category) showed largest differences in bilateral IFG with higher mean amplitudes for the phonologic compared with semantic fluency (channel 3, $t(324) = 9.5$; $p < 10^{-18}$; $d = 0.43$).

In order to investigate effects of lateralization, we contrasted mean amplitudes of corresponding channels of the left against channels of the right hemisphere (Fig. 5). The left hemisphere showed stronger activation within inferior orbital frontal areas and IFG (peak difference: channel 4; Letter–Weekday: $t(324) = 5.0$; $p < 10^{-5}$; $d = 0.33$; Category–Weekday: $t(324) = 4.1$; $p < 10^{-4}$; $d = 0.12$), channel 8, channel 12, whereas the postcentral gyrus (channel 20; Letter–Weekday: $t(324) = -3.2$; $p < 0.01$; $d = 0.20$; Category–Weekday: $t(324) = -3.4$; $p < 10^{-3}$; $d = 0.03$) and MFG (channel 18 [Letter–Weekday: $t(324) = -3.3$; $p < 0.01$; $d = 0.19$; Category–Weekday: $t(324) = -2.7$; $p < 0.01$; $d = 0.04$; not significant after FDR correction and $p < 0.01$ threshold]), channel 22) were more activated in the right hemisphere.

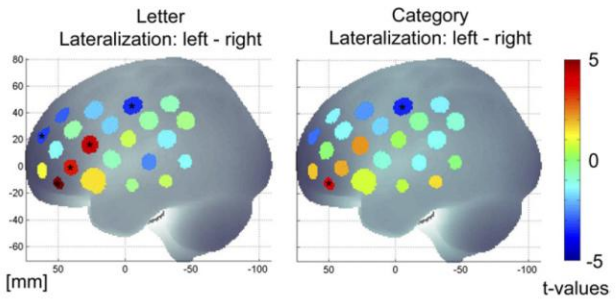

Fig. 5. (a) Letter–Weekday and (b) Category–Weekday mean signal amplitudes of left hemispheric functional near-infrared spectroscopy (fNIRS) channels contrasted with corresponding right hemispheric channels (left–right). Asterisks indicate significant differences (FDR-corrected; $p < 0.01$).

3.3. fNIRS data and age

Correlations of the mean amplitudes of the Letter–Weekday and the Category–Weekday contrasts, respectively, showed similar patterns of correlation (Fig. 6). Bilateral IFJ (channel 3: Letter–Weekday: left: $r = -0.18$; $p < 0.001$; $d = 0.37$; right: $r = -0.17$; $p < 0.01$; $d = 0.35$; Category–Weekday: left: $r = -0.23$; $p < 0.001$; $d = 0.47$; right: $r = 0.18$; $p < 0.01$; $d = 0.37$) as well as inferior frontal and middle temporal regions showed negative correlations between the participants' age and mean hemodynamic response amplitudes, i.e., a decrease in activation with increasing age. In turn, fNIRS channels with small O_2–Hb signal increases (relative decreases) showed positive correlations with age within bilateral inferior parietal regions, e.g., supramarginal gyrus (channel 14: Letter–Weekday: left: $r = 0.19$; $p < 0.001$; $d = 0.39$; right: $r = 0.16$; $p < 0.01$; $d = 0.32$; Category–Weekday: left: $r = 0.17$; $p < 0.01$; $d = 0.35$; right: $r = 0.18$; $p < 0.01$; $d = 0.37$) as well as MFG (e.g., channel 21: Letter–Weekday: left: $r = -0.18$; $p < 0.01$; $d = 0.37$; right: $r = 0.23$; $p < 0.001$; $d = 0.47$; Category–Weekday: left: $r = 0.21$; $p < 0.001$; $d = 0.43$; right: $r = 0.17$; $p < 0.01$; $d = 0.35$). The spatial pattern and magnitudes of correlations were comparable using nonmean corrected data.

To investigate the predictive value of age, sex, task performance, and years of education on the lateralization of cortical activation (left–right contrast), we used a multiple regression analysis of channels showing significant lateralization effects. Within inferior frontal gyrus (channel 8) age ($\beta = -0.11$; $p < 0.1$) and sex ($\beta = 0.11$; $p < 0.1$) showed a trend toward significant prediction in the multiple regression model ($F(4,321) = 2.48$; $p < 0.05$) of phonologic fluency (Letter–Weekday). Thus, with increasing age the left lateralization is marginally reduced and males exhibit a marginally pronounced left lateralization compared with females. For all other channels and condition contrasts the multiple regression of lateralized activation was not significant.

In order to investigate general/systemic changes in vascular reactivity with increasing age, we averaged the

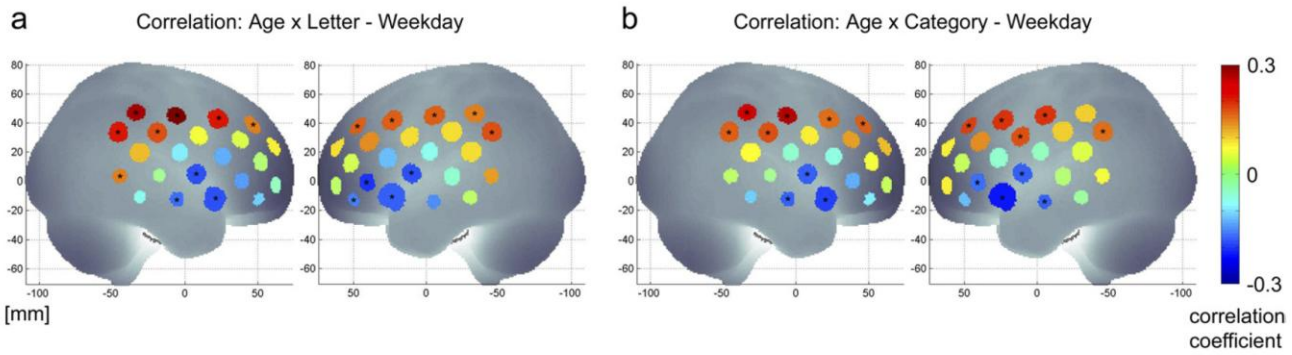

Fig. 6. Topography of correlation coefficients of the association between changes in oxygenation and increasing age of the participants. (a) Letter–Weekday and (b) Category–Weekday signal contrasts. Asterisks indicate significant correlations (FDR-corrected; $p < 0.01$).

mean event-related hemodynamic response amplitudes or condition contrasts over all channels of a probe set. None of the probe set responses were correlated with age ($p > 0.1$).

3.4. Multiple regression of fNIRS activation

Within the regions IFJ, MFG, and supramarginal gyrus, peak correlations between fNIRS channel activation and age were further analyzed using a multiple regression approach to assess the predictive value of age as well as sex, years of education, and task performance. In total, twelve multiple regression models were calculated, which explained between 3% and 10% of the variance (R^2) in the fNIRS data (Table 1).

In line with previously calculated correlations, increasing age predicted decreased activation within bilateral IFJ and increased activation within bilateral MFG and supramarginal gyri for both VFT task conditions. Simultaneously, sex predicted phonologic fluency activation with females showing increased bilateral IFJ activation compared with males, and males showing a statistical tendency toward relatively increased left MFG and left supramarginal gyrus activation. Also, years of education predicted bilateral IFJ phonologic fluency activation with longer education being associated with increased activation. Moreover, while longer education was a predictor for decreased right MFG and right supramarginal gyrus activation during semantic fluency, longer education showed a tendency toward decreased left MFG activation during phonologic fluency. VFT task performance, however, was not a significant predictor of fNIRS activation in the multiple regression models. All predictors showed small effect sizes ($-0.24 < \beta < 0.22$).

4. Discussion

Investigating the largest cohort so far using fNIRS, we were able to provide further evidence for aging-related alterations of cognitive processing in individuals aged 51 to 82 years. In this cohort, increased age predicted decreased hemodynamic responses within bilateral IFJ and increased bilateral MFG and supramarginal gyri responses elicited by phonological and semantic verbal fluency. Both longer education and grouping by sex (females > males) were significant predictors for increased bilateral IFJ activation during phonologic fluency. Additionally, males showed a tendency toward relatively increased left MFG and left supramarginal gyrus phonologic fluency activation. Moreover, longer education was a predictor for decreased right MFG and right supramarginal gyrus activation during semantic fluency. VFT task performance, however, was not a significant predictor of fNIRS activation. All predictors of the multiple regression models had small effect sizes ($-0.24 < \beta < 0.22$), while 3% to 10% of variance in the fNIRS data were explained by the models. Both experimental conditions showed higher activation of left compared with the right inferior frontal regions, whereas postcentral and MFG were more activated in the right hemisphere. However, for the inferior frontal cortex lateralization effects age was only a marginally significant predictor indicating reduced left lateralization of phonological fluency activation with increasing age.

In line with previous findings of reduced inferior frontal activation during the VFT in elderly compared with young subjects (Herrmann et al., 2006), the present results show this negative association in a large cohort of subjects aged 51–82 years. Moreover, we revealed positive associations of age and task-related activation within the bilateral middle frontal and inferior parietal gyri, which may reflect neural signatures of reorganization of cognitive function, or, in other words, compensation strategies. Negative associations of age and activation within inferior frontal regions showed small effect sizes ($\beta = -0.11$ to -0.21) for both hemispheres. Similarly, for positive associations the effect size β ranged between 0.15 and 0.22 for bilateral MFG and between 0.15 and 0.21 for the supramarginal gyri.

In this regard, two major aging theories on neural reorganization and compensation of a variety of cognitive functions have been proposed: The hemispheric asymmetry reduction in older adults (HAROLD) model (Cabeza, 2002) and the posterior-anterior shift in aging (PASA) model

Table 1
Multiple regressions of cortical activation during phonological and semantic fluency

Criterion variable: fNIRS activation			Predictor variable			
			Age			
Condition	Anatomic label	Channel number	Impact	β	B	SE(B)
Phonological fluency (Letter–Weekday)	IFJ (left)	3	(−)	−0.107(*)	−0.0021	0.0011
	MFG (left)	21	(+)	0.151**	0.0013	0.0005
	Supramarginal gyrus (left)	14	(+)	0.147*	0.0011	0.0004
	IFJ (right)	3	(−)	−0.138*	−0.0026	0.0011
	MFG (right)	18	(+)	0.218***	0.0015	0.0004
	Supramarginal gyrus (right)	14	(+)	0.207***	0.0018	0.0005
Semantic fluency (Category–Weekday)	IFJ (left)	3	(−)	−0.204***	−0.0036	0.0010
	MFG (left)	21	(+)	0.191***	0.0014	0.0004
	Supramarginal gyrus (left)	14	(+)	0.149*	−0.0009	−0.0003
	IFJ (right)	3	(−)	−0.180**	−0.0030	0.0009
	MFG (right)	18	(+)	0.140*	0.0009	0.0004
	Supramarginal gyrus (right)	14	(+)	0.170**	0.0010	0.0003

The fNIRS activation predictors age, sex, and years of education are displayed regarding the standardized regression coefficient (β; predictor effect size), the regression coefficient B and its standard error, SE(B). R^2 of the full regression model and analyses of variance statistics ($F(4,321)$ values) are shown. Sex is entered as a dummy variable with females = 0 and males = 1. The task performance was not significant as predictor in the multiple regression models. While only significant values are shown, asterisks indicate p values as $p < 0.1$(*), $p < 0.05$*, $p < 0.01$** and $p < 0.001$***.
Key: IFJ, inferior frontal junction; MFG, middle frontal gyri.

(Davis et al., 2008). Accordingly, neural compensation of cognitive function may occur through more bilateral prefrontal recruitment or through activation of prefrontal areas in addition to (posterior) regions normally involved in task processing without or with less prefrontal involvement. The present study found inferior frontal and frontotemporal areas to be (highly) significantly activated in both hemispheres. While inferior frontal areas of the left hemisphere were more engaged in verbal fluency processing as compared with corresponding right-hemispheric regions, the difference in activation (left–right) was only marginally reduced with increasing age. Here, the already bihemispheric activation during verbal fluency may render aging-related compensation, as predicted by the HAROLD model, less efficient. Also, participants of the present study were older than 51 years and effects predicted by the HAROLD model might be more pronounced in comparison with a group of young individuals. With respect to the PASA model, the relatively increased engagement of dorsolateral parts of the prefrontal cortex with increasing age is partly in line with findings of increased prefrontal activation in elderly compared with young subjects performing tasks of working memory, visual attention, and episodic retrieval (Cabeza et al., 2004; Spreng et al., 2010). Here, increased prefrontal processing was interpreted as higher order cognitive processing compensating for aging-related deficits in sensory processing. In contrast to studies investigating PASA-related effects, sensory input was minimal (eyes closed, short verbal task instructions) in the present study. However, while the exact mechanisms by which increased (dorsolateral) prefrontal engagement may compensate aging-related deficits remain speculative, for instance, MFG activation as identified by the present study may reflect increased cognitive control or attention, which may also partly underlie the PASA-related compensation.

Quantitative meta-analytic analyses of fMRI studies investigating different aspects of executive functions recently showed that bilateral IFG, MFG, and inferior parietal cortex represent large parts of a superordinate fronto-cingulo-parietal cognitive control network, which is involved in executive domains, such as flexibility, inhibition, initiation, and working memory (Niendam et al., 2012). Thus, relatively increased activity in MFG and inferior parietal cortex (comprising the supramarginal gyri) is functionally plausible to represent compensation mechanisms of decreased verbal fluency processing underlying effortful word retrieval. Preventing aging-related decline in verbal fluency performance, enhanced function of executive domains, such as flexibility and inhibition, may functionally compensate decreased neural IFG recruitment. Because the inferior frontal gyrus is also part of the cognitive control network, the compensatory activation within MFG and inferior parietal cortex may either reflect reorganization of these executive functions or additional activation subserving cognitive functions to compensate for decreased cognitive components of effortful and strategic phonological or semantic word retrieval processed within IFJ. Moreover, the role of the bilateral supramarginal gyri for phonological decisions (Hartwigsen et al., 2010) suggests increased activity within this region to represent a compensation mechanism specifically involving language-related processing. An alternative interpretation arises from findings showing that dorsolateral prefrontal and superior parietal areas are part of the neural attention network (Bush and Shin, 2006). Thus, increased activation with increasing age might indicate elevated attention levels during performance of the VFT, perhaps in an

Table 1
(Continued)

Sex					Years of education				Multiple regression model	
Channel number	Impact	β	B	SE(B)	Impact	β	B	SE(B)	R^2	F value
3	Female > male	−0.241***	−0.0718	0.0174	(+)	0.156*	0.0076	0.0030	0.101	8.95***
21	Male > female	0.111(*)	0.0143	0.0077	(−)	−0.106(*)	−0.0023	0.0013	0.049	4.12**
14	Male > female	0.102(*)	0.0122	0.0072					0.050	4.16**
3	Female > male	−0.170**	0.0489	0.0170	(+)	0.146*	0.0069	0.0030	0.074	6.33***
18									0.053	4.44**
14									0.064	5.44***
3									0.067	5.70***
21									0.045	3.71**
14									0.034	2.78*
3									0.048	4.01**
18					(−)	−0.134*	−0.0021	0.0009	0.042	3.45**
14					(−)	−0.120*	−0.0018	0.0009	0.051	4.22**

effort to compensate for aging-related functional deficits in attention as well as in verbal fluency.

Compared with age- and sex-matched healthy controls, AD patients have been shown to exhibit decreased bilateral inferior frontal cortex activation during phonological and semantic verbal fluency (Herrmann et al., 2008). Thus, decreased IFJ activation might hold predictive value indicating a trajectory toward AD diagnosis. While in healthy subjects VFT-related activation was not correlated with behavioral performance (Herrmann et al., 2003; Reif et al., 2011), AD patients exhibit negative associations of IFJ metabolism and lateral prefrontal regional cerebral blood flow, respectively, with semantic VFT performance (Kitabayashi et al., 2001; Schroeter et al., 2012). Also, compared with healthy controls, bilateral dorsolateral prefrontal cortex of MCI patients has been shown to be functionally disconnected from inferior parietal lobule and thalamus during resting state (Liang et al., 2011). Here, the degree of disconnection was correlated with declining performance in several cognitive tasks. Thus, both decreased IFJ as well as increased MFG and supramarginal gyrus engagement might serve to refine the prediction of deficits in behavioral performance as present in MCI and AD.

Using fMRI a group comparison showed increased right MFG and right inferior frontal gyrus activation in elderly (mean: 69.3 ± 5.6 years) compared with young individuals (26.1 ± 3.7 years) during semantic fluency (Meinzer et al., 2009). Complementing these findings, we show that also within a cohort of elderly subjects (64.6 ± 7.3 years) age is a significant predictor of increased MFG activation during verbal fluency. However, in the study by Meinzer et al. (2009) this additional activation in elderly individuals (n = 16) was correlated (r = −0.6) with behavioral performance, while for the phonological condition no group differences in activation or correlations with performance were present. In contrast to these findings, task performance showed no significant predictive value regarding fNIRS activation in the present fNIRS study. In our sample, age was negatively correlated with the semantic (r = −0.13), but not with phonological VFT performance, which is consistent with previous findings in healthy elderly subjects and with AD patients exhibiting more impaired semantic, but not phonological fluency, compared with measures of verbal intelligence and psychomotor speed (Henry et al., 2004). The effortful retrieval of words involves behavioral strategies, such as clustering of words from one category within a phonological or semantic task condition and switching between categories, i.e., the effective search process of finding new categories (Troyer et al., 1997). Differences in these strategies might also contribute to age-as well as sex-related behavioral and neural differences. Elderly compared with young subjects have been shown to exhibit relatively decreased switching, especially in semantic fluency (Lanting et al., 2009), and the production of fewer words might reflect a decline in executive function contributing to decreased IFJ activation with increasing age. Similarly, elderly female compared with male subjects have been shown to employ more switching in phonemic as well as semantic fluency (Lanting et al., 2009). Thus, better VFT performance (especially in semantic fluency) as well as increased left IFJ activation during phonemic fluency in females compared with males might be a consequence of differing behavioral strategies.

In line with previous studies (Birn et al., 2010; Ehlis et al., 2007; Herrmann et al., 2006; Schecklmann et al., 2008), we were able to show pronounced activation of left compared with right inferior frontal areas as well as higher IFJ activation during phonological compared with semantic fluency. In contrast to the HAROLD model (Cabeza, 2002), which was derived from findings related to memory and inhibitory processes, we found age to only marginally predict the relative engagement of left compared with right IFJ during (phonological) verbal fluency in healthy, non-demented elderly subjects. However, effect sizes of neural

reorganization, as indicated by increased MFG and supramarginal gyri activation with increasing age, were larger in the right compared with the left hemisphere, suggesting that the right hemisphere is relatively more involved in aging-related reorganization of verbal fluency processing. This might indicate that compensation via different executive domains processed within the cognitive control network may be relatively more engaged in the right hemisphere.

Limitations of the current findings have to be considered that might have partly contributed to the small effect sizes of the impact of age, sex, years of education and task performance on functional activation. First, functional hemodynamic responses as measured using fNIRS or fMRI only indirectly mirror neural activity underlying brain function. Thus, in addition to neural alterations, changes in vascular function and neurovascular coupling might partly contribute to aging-related effects on functional hemodynamics. However, we found both negative as well as positive associations of age and functional hemodynamic responses within specific cortical regions implicated in cognitive, executive, and language processing. Moreover, because the event-related hemodynamic response amplitudes averaged over the entire probe set were not correlated with age, it is unlikely that a general or systemic aging-related decline in vascular reactivity with increasing age underlies the present findings. Additionally, because our findings are (partly) in line with positron emission tomography data showing IFJ glucose hypometabolism to underlie AD-related deficits in verbal fluency performance (Schroeter et al., 2012), aging-related vascular effects are unlikely to critically underlie the present findings. Second, changes in skin blood flow have been argued to modulate fNIRS signals elicited by a phonologic VFT (Takahashi et al., 2011). To account for potential confounders, such as changes in skin blood flow, we corrected the channel-wise time series by subtracting the mean (systemic) response of all fNIRS channels at each sampling point (every 0.1 seconds). Moreover, to eliminate arousal confounders and (functional) hemodynamics related to word production, we contrasted mean amplitudes of experimental with the control condition, wherein a comparable number of words were verbally articulated. With these procedures blood flow changes within the skin can be largely excluded as a factor masking the functional hemodynamics within the cortical gray matter of interest. Third, the path length of near-infrared light and fNIRS sensitivity of continuous wave systems is dependent on the scalp-to-cortex distance and underlying tissue composition at a specific channel position (Haeussinger et al., 2011). Thus, comparisons of functional hemodynamic responses measured by fNIRS channels of the left and the right hemisphere might be influenced by local and hemispheric anatomic differences. Moreover, aging-related reductions in functional hemodynamics might not be associated with reduced neural processing per se, but could also be a consequence of neurodegeneration and cortical atrophy impacting fNIRS sensitivity. However, similar lateralization effects as well as aging-related decline in IFJ activity have been repeatedly shown using other neuroimaging techniques (Birn et al., 2010; Meinzer et al., 2009; Ravnkilde et al., 2002). Future studies should, however, address the impact of anatomic differences due to neurodegeneration on the sensitivity of functional imaging methods.

In conclusion, the present fNIRS study replicates results from previous studies with smaller samples reporting an aging-related decline in bilateral IFJ activity during verbal fluency. Moreover, the study revealed bilaterally increased activity in the bilateral MFG and supramarginal gyri as a correlate of aging-dependent compensation of cortical verbal fluency processing. The present study suggests the consideration of sex differences in activation. Additionally, one may speculate that the cortical reorganization of activation with increasing age might reveal neural changes due to AD-associated neurodegeneration occurring before behavioral and cognitive deficits become apparent. Thus, fNIRS may represent a useful tool for the investigation of antecedent neural markers of neurodegeneration, which may ultimately aid an individual early diagnosis of AD. Longitudinal, large, and multidisciplinary cohort studies, such as the TREND study, will have to address this issue in the future.

Disclosure statement

None of the authors reports any financial interests or potential conflicts of interest.

The study was approved by the ethical committee of the Medical Faculty of the University of Tuebingen (Nr. 90/2009BO2). All procedures were in accordance with the Declaration of Helsinki in its latest version, and all subjects gave written informed consent.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2012.05.021.

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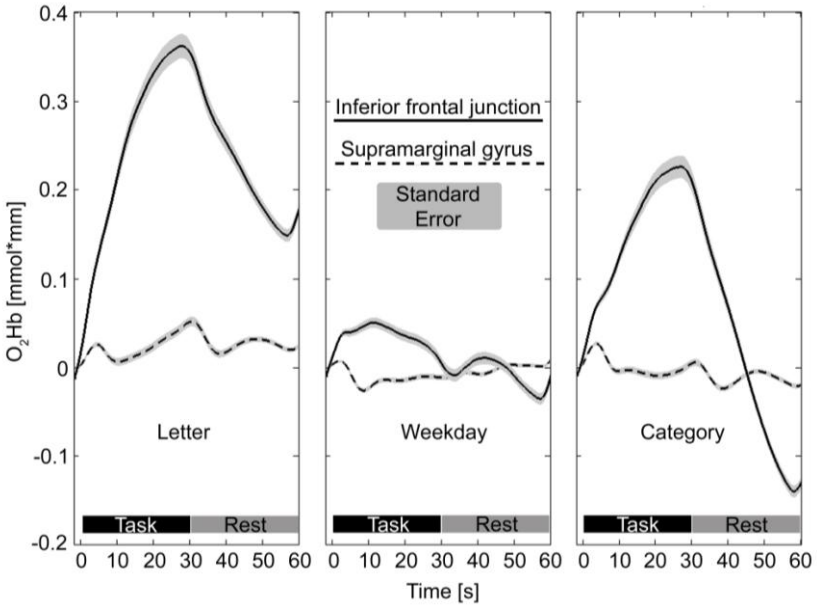
Study #2: Online supplementary data and information

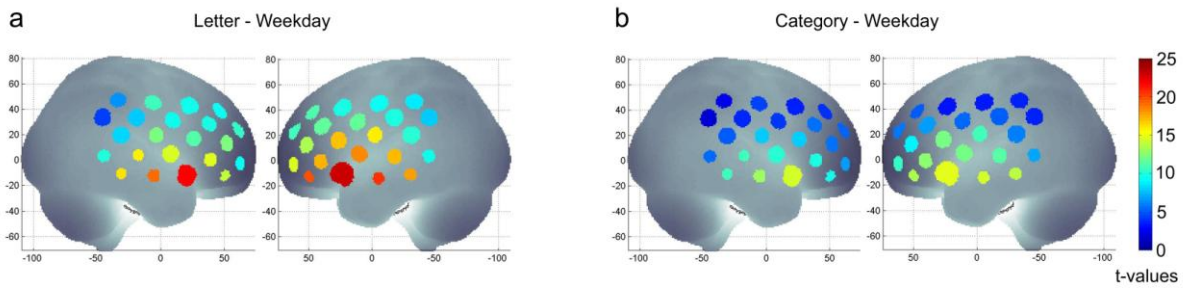
fNIRS data analyses (O_2Hb) without mean-correction

Without mean correction all channels showed O_2Hb signal increases. The event-related averages of the non-corrected O_2Hb signal of channels of maximal and minimal amplitudes, respectively, are shown in Supplementary figure 1.



Supplementary figure 1: O_2Hb data event-related average of channels showing maximal (left ch#3; Inferior frontal junction) and minimal (left ch #14; supramarginal gyrus) mean amplitudes, respectively.

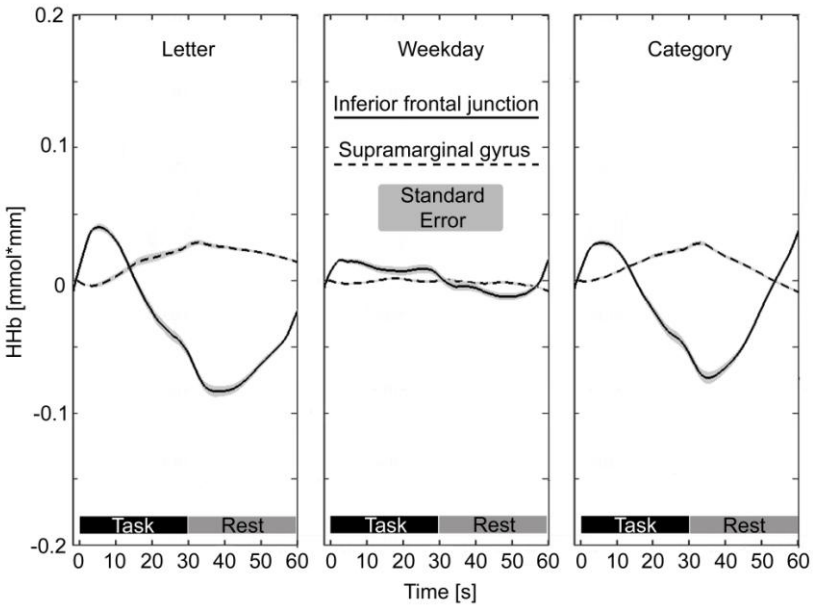
The general topography of channel-wise mean hemodynamic response amplitudes did not considerably differ between analyses with and without mean-correction (see Supplementary figure 2). However, without this method the t-value of mean amplitudes of each channel was positive and higher compared to the mean-corrected data. Note, that this correction method had little effect on the magnitudes and significance of the correlation between fNIRS channel mean amplitudes and age.



Supplementary figure 2: T-value maps of O_2Hb data without mean-correction. a) Phonological condition contrasted with the control condition. b) Semantic condition contrasted with the control condition.

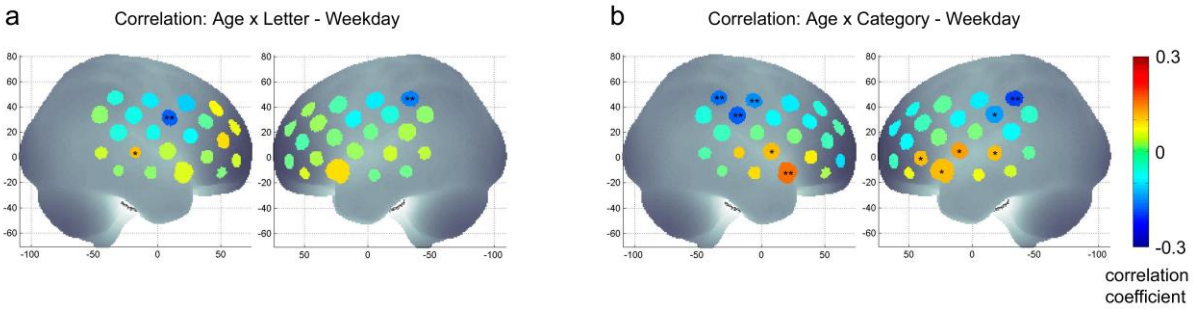
HHb data analyses

The event-related averages of the non-corrected HHb signal of channels showing maximal and minimal, respectively, amplitudes for the O_2Hb signal are shown in Supplementary figure 3.



Supplementary figure 3: Event-related averages of HHb data show, that after an initial HHb signal increase pronounced decreases during the phonological and semantic task intervals become apparent.

Channel-wise correlations of mean-corrected HHb data with the participants' age (Supplementary figure 4).



Supplementary figure 4: The topography of correlation coefficients (r) show, that while for the Letter-Weekday contrast correlations are only partly complementary to the corresponding O_2Hb analyses, the correlations with the Category-Weekday mirror findings of O_2Hb data. Asterisks indicate significant correlations ($p<.05$; FDR-corrected) and double asterisks indicate highly significant correlations ($p<.01$; FDR corrected).

Cumulative dissertation of Dipl.-Biol. Sebastian Heinzel:
Declaration of own and co-author contributions

Manuscript:

"Variability of (functional) hemodynamics as measured with simultaneous fNIRS and fMRI"

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Organization and participant recruitment: SH, AJF
fNIRS and fMRI data acquisition: SH, FBH
Programming of analysis tools: SH, FBH, TH
Data analysis and interpretation: SH, FBH, TH, ACE
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Study #3

Variability of (functional) hemodynamics as measured with simultaneous fNIRS and fMRI

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Abstract

Neural processing inferred from hemodynamic responses measured with functional near infrared spectroscopy (fNIRS) may be confounded with individual anatomical or systemic physiological sources of variance. This may hamper the validity of fNIRS signal interpretations and associations between individual traits and brain activation, such as the link between impulsivity-related personality traits and decreased prefrontal cognitive control during reward-based decision making.

Hemodynamic responses elicited by an intertemporal choice task in 20 healthy subjects were investigated for multimodal correlations of simultaneous fNIRS-fMRI and divergence in associations with trait "sensitivity to reward" (SR). Interindividual and interregional differences in anatomy and scalp fMRI signal fluctuations were correlated with fNIRS signals.

While showing substantial individual variability, the temporal fNIRS-fMRI correlations increased with the activation, which both methods consistently detected within right inferior/middle frontal gyrus. Here, up to 41% of fNIRS channel activation variance were explained by individual gray matter volume simulated to be reached by near-infrared light, and up to 20% by scalp-cortex distance. Extracranial fMRI and fNIRS time series showed significant temporal correlations in the temple region. SR was negatively correlated with fMRI but not fNIRS activation elicited by immediate rewards of choice within right inferior/middle frontal gyrus. Higher SR increased the correlation between extracranial fMRI signal fluctuations and fNIRS signals.

Task-related fNIRS signals might be impacted by regionally and individually weighted sources of anatomical and systemic physiological error variance. Trait-activation correlations might be affected or biased by systemic physiological responses, which should be accounted for in future fNIRS studies of interindividual differences.

Introduction

Functional magnetic resonance imaging (fMRI) and functional near-infrared spectroscopy (fNIRS) measure hemodynamic responses elicited by diverse stimuli and tasks. From these surrogate measures the functional neural processing is inferred, and conclusions regarding basic brain (dys)function as well as the basis of personal individuality are commonly drawn. However, specific merits and limitations are inherent to these functional neuroimaging methods. Specifically, fNIRS is – due to its cost-efficiency, practicability and high ecological validity – suitable for many clinical (Irani et al., 2007; Linden and Fallgatter, 2009), developmental (Lloyd-Fox et al., 2010) or language neuroimaging studies (Dieler et al., 2012), as well as for studies requiring large sample size (Heinzel et al., 2012). Moreover, fNIRS can be combined with various other concurrent modalities such as EEG, MEG or fMRI. However, anatomical and systemic physiological influences may hamper valid interpretations of fNIRS signals, i.e. blood oxygenation level-dependent (BOLD) responses, for the purpose of individually quantifying neural activation (Cui et al., 2011; Haeussinger et al., 2011; Kirilina et al., 2012; Takahashi et al., 2011). Since these influences may be individually-weighted correlations between activation measures and individual factors, such as personality traits, they may be affected or biased generating inconsistencies between fNIRS and fMRI findings.

The sensitivity of fNIRS measurements of neural correlates of specific cortical brain functions may be impacted by two major sources of error variance contributing to interindividual and inter-channel differences in fNIRS signals, and inconsistencies in regard to findings from other neuroimaging modalities: First, hemodynamic fluctuations in the scalp may be evoked by task-related arousal and systemic physiological artifacts in the forehead scalp or near the temple region (Gregg et al., 2010; Kirilina et al., 2012; Sato et al., 2011; Takahashi et al., 2011). Second, while several photon migration simulation studies showed that near-infrared light theoretically reaches the cortical gray matter of interest (Custo et al., 2006; Hoshi et al., 2005; Okada et al., 1997), the volume of gray matter traversed by the light was shown to be reduced in the frontal sinus region, to be negatively correlated with the scalp-cortex distance

(SCD) and to show substantial interindividual variability (Haeussinger et al., 2011). Functionally, temporal correlations between fNIRS and fMRI signals have been shown to be impacted by SCD and to have wide inter-subject and inter-channel variability (Cui et al., 2011). These anatomical and/or physiological influences on fNIRS signals may be individually weighted for each subject and/or region as well as dependent on the task characteristics regarding elicited regional functional activation and task-evoked arousal. Thus, group level activation as well as correlations of fNIRS signals with individual factors such as personality traits may, for some fNIRS experiments, lack robustness against this error variance. Following studies investigating the relation of fMRI and fNIRS measures of the BOLD response, we seek to assess the impact of the divergence between the two technologies on commonly computed quantities such as BOLD-personality correlations.

To address this issue, we conducted a simultaneous fNIRS-fMRI study using a reward-based decision making paradigm for which we hypothesized, (1) similar fNIRS and fMRI prefrontal group activation associated with cognitive control, which (2) is modulated by trait "sensitivity to reward" (SR). Moreover, aforementioned sources of individual and interregional anatomical and (systemic) physiological error variance were investigated regarding their influence on fNIRS signals and trait SR correlations.

We used an intertemporal choice (ITC) paradigm, in which monetary reward options of choice are offered which differ in delay to delivery and reward magnitude, i.e. smaller but sooner versus larger but later rewards are presented. ITC involves several sub-processes such as reward valuation, cognitive control and prospection, which account for the interindividual variability in neural and behavioral ITC outcomes (Peters and Buchel, 2011). The task was used to compare the expected interindividual prefrontal processing differences between fNIRS and fMRI, and the respective activation variance explained by trait "sensitivity to reward" (see below). ITC processes involve emotion-related reward processes which have been linked to fMRI activation within structures of the limbic motivation system (β-system), such as ventral striatum and ventro-medial prefrontal cortex, and executive functions mediated by regions (δ-system), such as the dorsolateral prefrontal cortex (DLPFC) and the posterior parietal cortex (Figner et al., 2010; Hare et al., 2009; McClure et al., 2007;

McClure et al., 2004). Similar to the findings by McClure et al. (2004) a previous fNIRS study using the same task reported, that the right DLPFC uniformly responds to immediate and delayed rewards, whereas the orbitofrontal cortex shows pronounced responses towards immediate rewards (Plichta, 2009). DLPFC and inferior PFC processing have repeatedly been shown to be involved in top-down cognitive control and the regulation of limbic reward regions involved in ITC, and reduced activation or dysfunction of these regions has been associated with more impulsive ITC behavior and with impulsivity-related psychopathologies (Goldstein and Volkow, 2011; Kim and Lee, 2011; McClure et al., 2004). Impulsivity is a multi-dimensional construct which is in part reflected by SR (Franken and Muris, 2006). Specifically, the personality trait SR is defined by the extent to which rewarding stimuli activate the behavioral approach system (BAS) mediating reactions to appetitive stimuli (Gray, 1991; Pickering et al., 1997). In addition to the role of SR for reward system processing (Hahn et al., 2011), dysfunction in top-down inhibition of reward system areas (Heatherton and Wagner, 2011) might partly underlie increased trait SR in disinhibitory disorders, such as ADHD or drug addiction (Franken et al., 2006; Mitchell and Nelson-Gray, 2006). Based on the functional role of the DLPFC for cognitive control during ITC and its regulatory role for impulsive behavior and limbic reward processing, we hypothesized subjects with high levels of trait SR to show relatively decreased DLPFC activation during ITC as measured with fNIRS and fMRI.

The present study investigated both methodological and functional factors which may modulate prefrontal fNIRS and fMRI hemodynamics elicited by ITC. (1) After comparison of the cortical group level activation, we assessed the interindividual and interregional variability of multimodal temporal correlations. (2) Variance in fNIRS activation explained by individual anatomy and scalp fMRI signals was quantified. (3) We investigated prefrontal functional ITC activation as measured with fNIRS and fMRI for consistency in regard to correlations with trait SR, and (4) anatomical and (systemic) physiological factors affecting fNIRS activation-trait associations.

Materials and Methods

Participants

A total of 24 adult healthy subjects participated in the simultaneous fNIRS-fMRI study. Three subjects (#5, #6, #14) were excluded due to noisy fNIRS data or motion artifacts as detected after visual inspection of the data. One additional subject (#17) was excluded due to overly long decision times (>30 s) during the ITC task. Data of 20 subjects (12 male, 4 left-handed, 1 regular smoker) with a mean age of 25.2 ± 2.9 years (± standard deviation; SD) were included in the analyses of the present study. The individual financial situation differed between participants as assessed by self-ratings on visual analog scales from 1 to 100 indicating (1) the subjective current financial situation (from "very bad" to "very good") (mean: 53 ± 25), and (2) how urgently they need 20 € at the moment ("not urgently" to "very urgently") (mean: 45 ± 30).

The anatomical data of the same subjects were previously analyzed and investigated to assess an impact of individual anatomy on near-infrared light reaching gray matter using Monte-Carlo simulations (Haeussinger et al., 2011). These data were also used for analyses of the present study.

All subjects were screened for any history of Axis I or II pathology, neurological disorders or psychoactive medication by questionnaires based on DSM-IV criteria for mental disorders. Two of the participants which were excluded due to motion artifacts reported a history of depressive episodes (#5) or migraine (#6), respectively, while none of the other participants reported any relevant pathologies or medication. All participants gave written informed consent. The study was in accordance with the latest version of the Declaration of Helsinki and approved by the Ethics Committee of the University of Wuerzburg.

Intertemporal Choice Task

During the simultaneous fNIRS-fMRI measurement a validated intertemporal choice paradigm (McClure et al., 2004; Plichta, 2009; Plichta et al., 2009) was used to elicit neural activation associated with the subjective valuation of the two monetary reward options of choice which differed in their amount and delay to delivery. Specifically, subjects were simultaneously presented an earlier but smaller reward option and a larger but (more) delayed reward option. In the 40 ITC trials, the amount of the early reward option ranged between 5 € and 40 € (mean amount 20 € ± 10€). Relative to the early option, the (more) delayed monetary amount was larger by either 1%, 3%, 5%, 10%, 15%, 25%, 35%, or 50%. The trials were assigned to "immediate" reward conditions (16 trials) if the early reward was delivered after the experiment, and to two "delayed" conditions if even the early reward option was delayed (2 weeks, 16 trails; or 4 weeks, 8 trials). Thus, depending on the individual reward option choice, money was delivered either today, after 2 weeks, or after 4 weeks in the "immediate" reward condition, whereas for the "delayed" reward condition money was delivered either 2 weeks or 4 weeks after the experiment. The intertemporal distance between the early and the late reward was either 2 weeks or 4 weeks.Reward options were presented and individual trial decision times and choices were recorded using presentation (Neurobehavioral Systems; http://www.neurobs.com).

While decision time was not constricted, participants were instructed to carefully weigh up each decision since one of the chosen reward options was randomly selected as payment for experiment participation. Monetary rewards were paid to the participants in cash after the experiment (today option) or delivered after the indicated delay via bank transfer.

The fNIRS and fMRI analyses were based on the event-related design of the ITC task using an intertrial interval of 14 s ± 2 s (black screen with white fixation cross).

Psychometric measures

We used a 48-item self-report inventory to assess individual differences in the personality trait of sensitivity to punishment (SP) and sensitivity to reward (SR) [SPSR questionnaire; (Torrubia et al., 2001); German version (Hewig and Hagemann, 2002)]. The SPSR questionnaire measures the two scales of SP and SR, for which 3-month retest reliabilities of 0.87 and 0.89, respectively, orthogonality, and good construct validity have been shown (Sava and Sperneac, 2006).

fMRI Acquisition

Structural and functional MRI was performed using a 1.5 T Siemens Magnetom Avanto TIM-system MRI scanner (Siemens, Erlangen, Germany) equipped with a standard 12-channel head coil. Structural images (T1-weighted) were recorded with a spatial resolution of 256 x 256 x 159 voxels with a voxel size of 0.98 mm x 0.98 mm x 1.00 mm using 3D magnetization prepared rapid gradient echo (MPRAGE) sequences with a repetition time (TR) of 1870 ms and an echo time (TE) of 3.74 ms. For functional MRI, we used a T2*-sensitive single-shot echo planar imaging (EPI) sequence with a 2-s TR, 40-ms TE, 90° flip angle and a 64 x 64 matrix within the field of view (210 mm x 210 mm). In a single session, 24 interleaved axial slices (4-mm thickness, 1-mm inter-slice gap in-plane resolution: 3.28 mm x 3.28 mm) oriented -30° (T<C) in respect to the AC-PC transverse plane were acquired. The first eight EPI volumes were discarded to account for magnetization saturation effects.

fMRI analysis

For fMRI analyses data were preprocessed and analyzed using statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, UK; implemented in Matlab 7.9, Math Works, Natick, MA, USA). For the preprocessing, motion and slice-time correction, and spatial normalization of the images was applied. The images

were spatially smoothed with an 8-mm full-width at half maximum (FWHM) Gaussian isotropic kernel. Time series data in each voxel was filtered (highpass: 1/128 Hz). For model-based regression analyses voxel-wise time series of each subject were modeled using three regressors, each indicating the temporal sequence of one type of ITC events (early reward option delivered: today; in 2 weeks; in 4 weeks). Events of each of these regressors were modeled as delta functions and convolved with a canonical hemodynamic response function. Also, the duration of each event was set to the mean decision time (3.7 s) of all trials and subjects. Differences in decision times were controlled for using a parametric regressor of trial-specific decision times. Additionally, regressors of motion parameters from preprocessing were included. Data were corrected for temporal autocorrelation using a first-order autoregressive model with a lag of 1. Parameters (β-weights) of each regressor were estimated using the general linear model. Effects of interest in each subjects were obtained by linear contrasts of the β-weights: "immediate" (today), 2 weeks, 4 weeks, "delayed" (mean of 2 weeks and 4 weeks), "delay discounting" contrast (today – delayed), and "all rewards" (mean of "immediate" and "delayed"). In the second level group analysis these voxel-wise β-weight contrasts were tested against zero using t-tests. For whole brain analyses we used family-wise error correction (FWE; $p < .05$) to account for multiple testing. The functional prefrontal region of interest (ROI) comprised voxels showing significant activation ("all rewards") using a more conservative FWE-corrected threshold of $p < .01$.

We correlated (Pearson correlation) the respective beta estimates with SR scores in a voxel-wise fashion yielding a correlation coefficient for each voxel. In order to assess significance of the results accounting for multiple comparisons, a permutation test based on cluster-sizes was conducted in our ROI as follows: First, we computed correlation maps between β-weights and SR scores for each voxel and thresholded the map at a single voxel p-value of 0.05. Then, we repeated this procedure using permuted SR scores 10,000 times. For each of the permutation runs, we assessed the size of the largest voxel cluster obtained at the same single voxel p-value. Clusters showing correlations between the β-weights and SR scores (single voxel p-value < .05) were considered significant when a cluster was larger than 95% of the cluster

sizes obtained under permutation. This ensures a corrected α-level of 5% on the cluster level (for a highly related procedure, see Nichols and Holmes, 2002).

fNIRS Acquisition

Relative changes in the concentration of oxyhemoglobin (oxy) and deoxyhemoglobin (deoxy), respectively, were recorded with an fNIRS continuous wave system (ETG-4000 Optical Topography System, Hitachi Medical Corporation, Tokyo, Japan) at a sampling rate of 10 Hz and an inter-optode distance of 3 cm. We used an MRI-compatible 4 x 4 probe array of 16 optodes comprising 8 semiconductor laser diodes (wavelengths: 695 ± 20 and 830 ± 20 nm) and 8 detectors. This setup contains 24 emitter-detector pairs (fNIRS channels) measuring the intensity changes in light traversing the tissues underneath the channel. For positioning of the probe set covering the right prefrontal cortex we used the International EEG 10-20 system. Specifically, the light emitter of NIRS-channel #1 and #4, respectively, was placed on Fpz while the bottom row of NIRS optodes of the probe set was located on a line from Fpz to F8. After positioning of the probe set we carefully placed MRI-compatible goggles (VisuaStim; Magnetic Resonance Technologies, Northridge, CA) ensuring both optimal probe set position and ITC reward option presentation. In order to minimize misalignment of the probe set and artifacts due to head movement, we used a cushioned head fixation device and rubber bands fixating the fNIRS probe set under the MRI-head coil. Since optodes showed skin indentations visible in the structural MRI, exact spatial optode positions could be individually identified. Individual optode positions on the head surface were normalized to *Montreal Neurological Institute* (MNI) space using SPM8 routines. The fNIRS channels were projected on the cortical surface yielding probabilistic values of anatomical labels using functions (http://www.jichi.ac.jp/brainlab/tools.html) of the *National Food Research Institute* (*NFRI*) in Japan (Singh et al., 2005). Automatic anatomical labels (AAL) of the highest probabilistic value of the neural structure underneath an fNIRS channel are reported.

fNIRS analysis

After applying a bandpass filter (1/128 Hz, 0.5 Hz) to the fNIRS data model-based regression analyses were conducted separately for oxy and deoxy fNIRS data using custom routines implemented in Matlab 7.9. Analyses were in close accordance with the fMRI analyses approaches using three ITC event regressors ("immediate", "2 weeks", "4 weeks") which were modeled as delta functions and convolved with a Gaussian hemodynamic response function. After visual inspection of the event related average over all channels and subjects, a peak time of 7 s and standard deviation 2.7 s was considered to provide a response function with the best fit to the data. The mean decision time of 3.7 s and an additional trial specific decision time weighted regressor was included for the β-weight estimation of fNIRS channel activation. Data were corrected for temporal autocorrelation using a first-order autoregressive model with a lag of 1 (Plichta et al., 2007). Bonferroni-corrections for 24 measurement channels (corrected threshold: $p < .002$) were used to account for multiple testing. For deoxy data and task condition contrasts ("delay discounting") also channel activation of an uncorrected threshold of $p < .01$ is reported. Moreover, event-related averages for the "immediate" and "delayed" task conditions were calculated. After baseline correction (subtraction of the mean signal between -0.5 s and 0 s) segments from 0 s (trial onset) to 16 s were averaged.
Note for all fNIRS activation and correlation analyses that neural activation is commonly followed by an increase in fNIRS oxy and a corresponding decrease in deoxy fNIRS signals resulting in opposite signs.

Anatomical measurements

In order to identify voxels reached by near-infrared light considering the individual anatomy underneath a given fNIRS channel, we previously employed Monte-Carlo simulations (see: Haeussinger et al., 2011). Briefly, we first performed automatic anatomic segmentation (SPM8 tool *New Segmentation*) of the structural MRI with a

voxel size of 0.96 mm³ to identify background (=air), scalp, scull, cerebrospinal fluid (CSF), gray matter and white matter voxels in the individual structural images. In a second step, we applied Monte-Carlo simulations of scattering and absorption processes of photon packages as near-infrared light traverses the individual tissues from a specific light emitter to the respective detector of an fNIRS recording channel. Approximately 5 x 10^6 simulations were conducted for each fNIRS channel in each subject of the present study. We, thereby, identified the gray matter voxels reached by the near-infrared light. For a direct comparison of fNIRS and fMRI signals, we first transformed the segmented images as well as the light absorption distributions from MPRAGE to EPI space. Subsequently, we extracted the mean fMRI EPI time series from the individual light absorbing volumes of gray matter (V_{gray}) and volumes of scalp (V_{scalp}) underneath each fNIRS channel and each subject. Moreover, we quantified the channel-wise scalp-cortex distance (SCD) and assessed the channel-wise Spearman correlation of V_{gray} and SCD, respectively, with the fNIRS activation magnitude (β-weights).

Temporal correlations of fNIRS with cortical and scalp fMRI signals

Interregional and interindividual differences in multimodal temporal correlations (Spearman) of the entire fNIRS and fMRI time series within V_{gray} and V_{scalp}, respectively, were quantified. First, the mean fMRI EPI time series from the (non-normalized, unsmoothed) individual cortical V_{gray} of each fNIRS channel was correlated with the corresponding fNIRS oxy and deoxy time series, respectively. Second, to assess the impact of (systemic) physiological artifacts on fNIRS signals, temporal correlations between the mean EPI fMRI time series in the scalp (V_{scalp}) underneath an fNIRS channel and the oxy and deoxy time series, respectively, were calculated. The coefficients obtained by the temporal correlations were then correlated with the fNIRS channel activation (β-weights, "all rewards"). For temporal signal correlations fNIRS data was downsampled to 0.5 Hz. Non-parametric Spearman correlations which are (compared to Pearson) more robust against potential outliers/artifacts

(and non-normality) were used for temporal correlations and correlations between anatomical measures and activation, whereas Pearson correlations were calculated for correlations between trait SR and activation. All Pearson and Spearman correlation coefficients at an α-level of 5% were considered statistically significant and marginally significant at an α-level of 10%.

Results

fMRI and fNIRS group activation

The intertemporal choice task ("all rewards") elicited significant fMRI activation (t-tests of β-weights across subjects, whole brain FWE-corrected, $p < .05$) in multiple areas. Major clusters of activation were found within the occipital-temporal lobe and cerebellum (14911 voxels), bilateral insula and inferior frontal gyrus (right: 371 voxels, left: 571 voxels), anterior and middle cingulate gyrus, medial frontal gyrus and bilateral supplementary motor area (1292 voxels), bilateral areas of the midbrain and brainstem including thalamus, pons, substania nigra and hippocampus (563 voxels), right caudate nucleus (35 voxels) as well as in the bilateral middle frontal gyrus (right: 534 voxels, left: 104 voxels) (Figure 1 and Figure 2a). The contrast between the "immediate" and "delayed" task activations showed no significant clusters after FWE correction ($p < .05$).

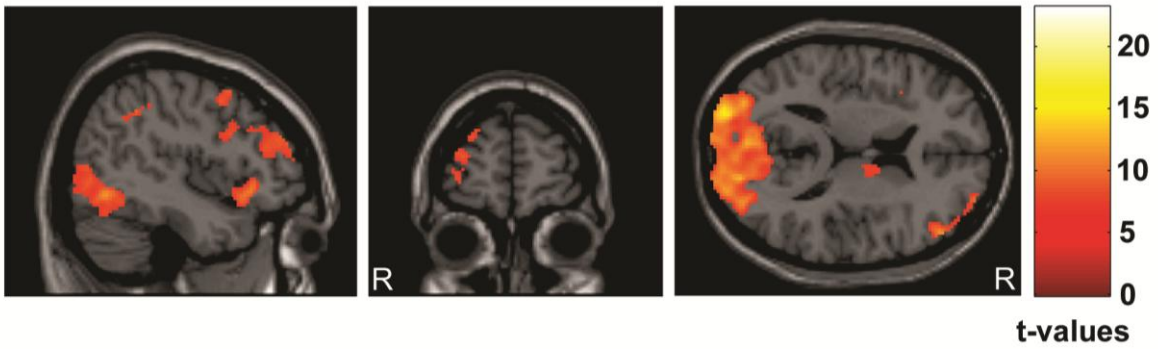

Figure 1

Intertemporal choice fMRI activation („all rewards", FWE-corrected, $p < .05$)

Model-based group analysis of the fNIRS oxy data showed a similar pattern of activation ("all rewards", t-tests of β-weights across subjects) in the right prefrontal cortex. Channels showing significant activation (oxy signal increase; Bonferroni-corrected for 24 recording channels; $p < .002$) were all labeled as right middle frontal gyrus (channel #2, 6, 9, 13, 17, 20, 24) except channel #10 (right inferior frontal gyrus, *triangular* part) (Figure 2b). The deoxy data showed a corresponding signal decrease. After Bonferroni-correction a significant decrease was observed within middle frontal gyrus (#10) and right superior frontal gyrus (#22). However, a more liberal threshold (uncorrected, $p < .01$) revealed a similar spatial activation pattern compared to oxy within right middle frontal gyrus (channel #2, 6, 13, 17, 20, 24) and right inferior frontal gyrus (#10) (Figure 2c). The "immediate" and "delayed" condition showed no significant difference in fNIRS activation (oxy, deoxy; uncorrected, $p < .01$). While the deoxy signal change was less pronounced the activation pattern showed more spatial specificity compared to the oxy signal. Moreover, deoxy, but not oxy, showed activation in the medial part of the superior frontal gyrus (#18, #22) in vincinity to the fMRI activation cluster comprising parts of the medial frontal gyrus, supplementary motor area and cingulate cortex. Neither oxy nor deoxy showed activation of channel #3 (inferior frontal gyrus, orbital part) underneath the temple region, where fMRI detected a significant activation cluster (inferior frontal gyrus/insula). However, both fNIRS (oxy and deoxy) and fMRI consistently showed a cluster of regional activation within right inferior/middle frontal activation during ITC (Figure 2a-c).

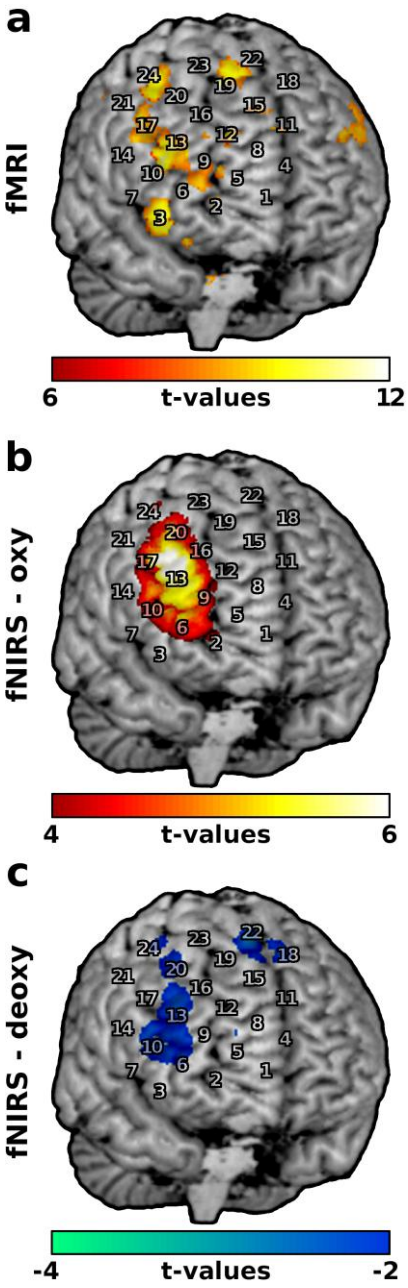

Figure 2

Multimodal comparison of group activation during intertemporal choice

a) Right prefrontal fMRI activation clusters ("all rewards", whole brain FWE-corrected, $p < .05$)

b) fNIRS activation topography of the oxy-signal ("all rewards", Bonferroni-corrected, $p < .002$)

c) fNIRS activation topography of the deoxy-signal ("all rewards", uncorrected, $p < .01$)

For illustration of the fNIRS and fMRI signals, standardized event-related averages with the standard errors of the mean (SEM) of the fNIRS oxy and deoxy signal, respectively, as well as the fMRI signal of the V_{gray} are shown for the peak oxy activation fNIRS channel (#13, right middle frontal gyrus; Figure 3).

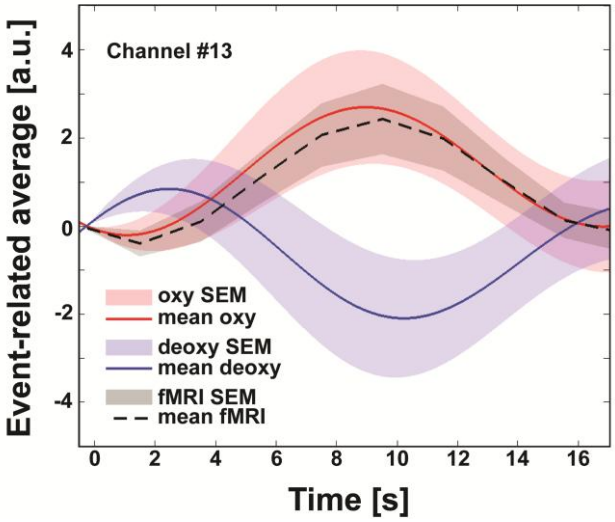

Figure 3

Event-related average ("all rewards") of the standardized fNIRS oxy- and deoxy-signal (channel #13, right middle frontal gyrus), and in the standardized mean fMRI EPI-signal (arbitrary units, a. u.) in gray matter voxels simulated to be traversed by the near-infrared light underneath the corresponding fNIRS optodes. Areas indicate the standard error of the mean (SEM) of the respective mean signal.

Temporal correlations of fNIRS and fMRI signals

To quantify interregional and interindividual differences in multimodal correlations (Spearman), fNIRS (oxy, deoxy) and fMRI time series signals within V_{gray} and V_{scalp}, respectively, were correlated. First, channel-wise temporal correlations between fNIRS oxy data and V_{gray} fMRI time series were calculated. The mean correlation coefficient r (over all subjects) for each of the 24 channels is shown in Figure 4a. Regarding all subjects and channels r ranged between .62 and -.43 with a mean

within-channel standard deviation (SD) of .19. The channel-wise coefficients were positively correlated ($r = .50$, $p < .05$) with the fNIRS channel activation (β-oxy, "all rewards"). For the deoxy and V_{gray} fMRI time series temporal correlation coefficients (Figure 4b) also showed a positive correlation ($r = .43$, $p < .05$) with the channel activation (β-deoxy, "all rewards"). The overall range of r was .60 to -.71 (mean within-channel SD: .21). Second, fluctuations of the fMRI signal in V_{scalp} were positively correlated with the fNIRS oxy time series, with highest correlations near the right temple and temporal muscle region ($r \leq .2$; channel #3, 7, 10, 14, 21) extending to fNIRS channels labeled as middle frontal gyrus ($r > .1$; #9, 13, 24) (Figure 4c). The highest correlation (#7, mean $r = .20 \pm .28$) had a range from -.28 to .74 between subjects, while coefficients were not correlated with the activation (β-oxy, "all rewards") ($p > .1$). For the deoxy signal, corresponding negative correlations (deoxy ~ V_{scalp} fMRI time series) showed a similar pattern compared to oxy. While the magnitude of negative correlations near the temple region was smaller ($r \geq -.09$), additional small positive correlations ($r \leq .12$) were present near the hemispheric cleft (Figure 4d). The highest negative correlation (#10, mean $r = -.09 \pm .22$; range: -.47 to .36) showed considerable interindividual variability. Also, r was not correlated with the activation (β-deoxy, "all rewards"). Due to the large number of about 300 data sampling points used for the temporal correlation of entire time series, reported correlations were (highly) significant.

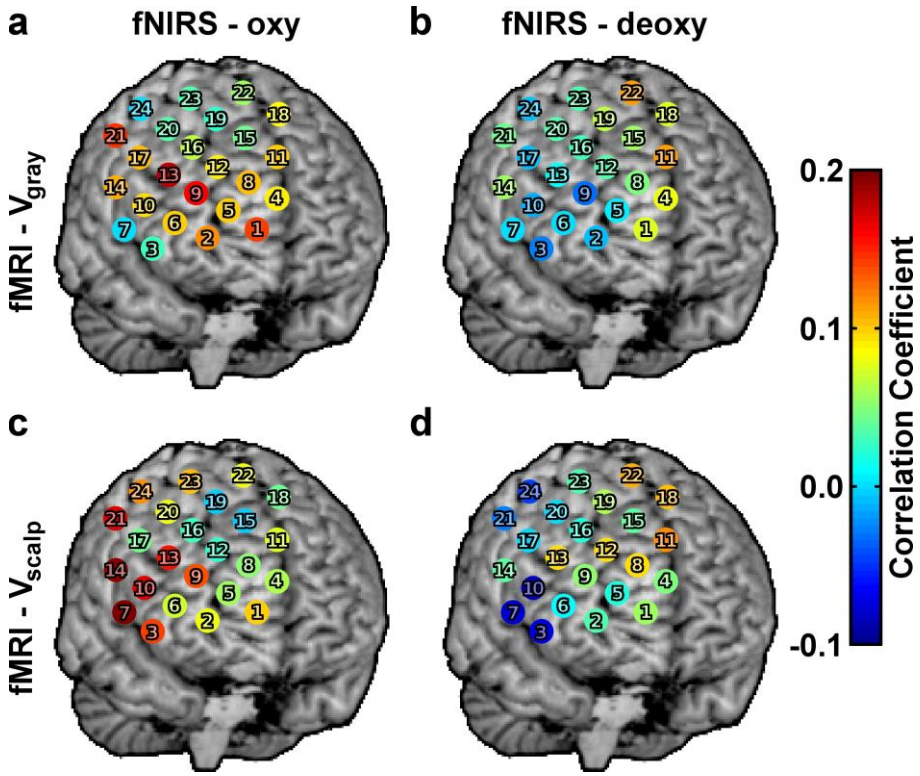

Figure 4

Temporal correlations (Spearman) of the signal time series between a) fNIRS oxy and fMRI in the corresponding volume of gray matter (V_{gray}), b) fNIRS deoxy and fMRI in V_{gray}, c) fNIRS oxy and fMRI scalp signals underneath the corresponding fNIRS channel, and d) fNIRS deoxy and fMRI scalp signals.

Anatomical influences on fNIRS sensitivity

In order to investigate the variance in fNIRS channel activation which is explained by individual anatomy, the fNIRS activation estimates (β-weights, "all rewards") were correlated with the individual channel-wise SCD and V_{gray}, respectively. Generally, SCD varied between channels from 12.6 ± 1.5 mm (mean ± SD of channel #10) to 17.5 ± 2.9 mm (#22). Similarly, V_{gray} ranged from 0.51 cm³ ± 0.46 cm³ (#18) to 1.82 cm³ ± 0.56 cm³ (#14). Channel mean values of SCD and V_{gray} (± inter-subject SD) are shown in Table 1. SCD was negatively correlated with V_{gray} (Pearson $r = -.72$, $p < .0001$).

Table 1:

Channel #	*Scalp-cortex distance (SCD) [mm] ± inter-subject SD*	*Gray matter volume (V_{gray}) [cm^3] ± inter-subject SD*
1	15.2 ± 2.1	0.80 ± 0.29
2	13.3 ± 2.4	1.11 ± 0.54
3	13.8 ± 2.2	0.94 ± 0.56
4	15.8 ± 2.5	0.96 ± 0.48
5	13.8 ± 2.3	1.37 ± 0.46
6	11.8 ± 1.6	1.22 ± 0.63
7	14.6 ± 1.7	1.54 ± 1.57
8	15.0 ± 2.0	0.95 ± 0.37
9	13.5 ± 2.0	1.35 ± 0.39
10	12.6 ± 1.5	1.47 ± 0.60
11	16.3 ± 1.9	0.65 ± 0.40
12	14.7 ± 2.0	0.93 ± 0.47
13	13.4 ± 1.9	1.32 ± 0.60
14	13.3 ± 1.7	1.82 ± 0.56
15	16.2 ± 2.6	0.80 ± 0.48
16	14.9 ± 2.3	0.89 ± 0.59
17	13.2 ± 1.9	1.41 ± 0.67
18	16.0 ± 3.1	0.51 ± 0.46
19	16.4 ± 2.4	0.66 ± 0.39
20	13.6 ± 2.5	0.83 ± 0.66
21	13.4 ± 2.1	1.66 ± 0.56
22	17.5 ± 2.9	0.74 ± 0.46
23	15.5 ± 2.4	0.99 ± 0.60
24	14.0 ± 2.1	1.16 ± 0.63

First, for the oxy data SCD showed a significant negative correlation with activation in channel #22 (r = -.74, p < .001) and #23 (r = -.58, p < .001). All other channel correlations were not significant (p > .1). Throughout the probe-set negative correlation coefficients prevailed (r range: .09 to -.74; mean r = -.19 ± .23; Figure 5a). Similarly, for the deoxy data (Figure 5b) channel #22 (r = .45, p < .05) showed a corresponding positive correlation between activation (β-deoxy, "all rewards") and SCD. Additionally, channel #2 (r = .53, p < .05), and the functional ROI channels #10 (r = .45, p < .05) and #13 (r = .51, p < .05) showed positive correlations with SCD. The correlation coefficient (β-deoxy ~ SCD) range was -.15 to .53 (mean r: .24 ± .19).

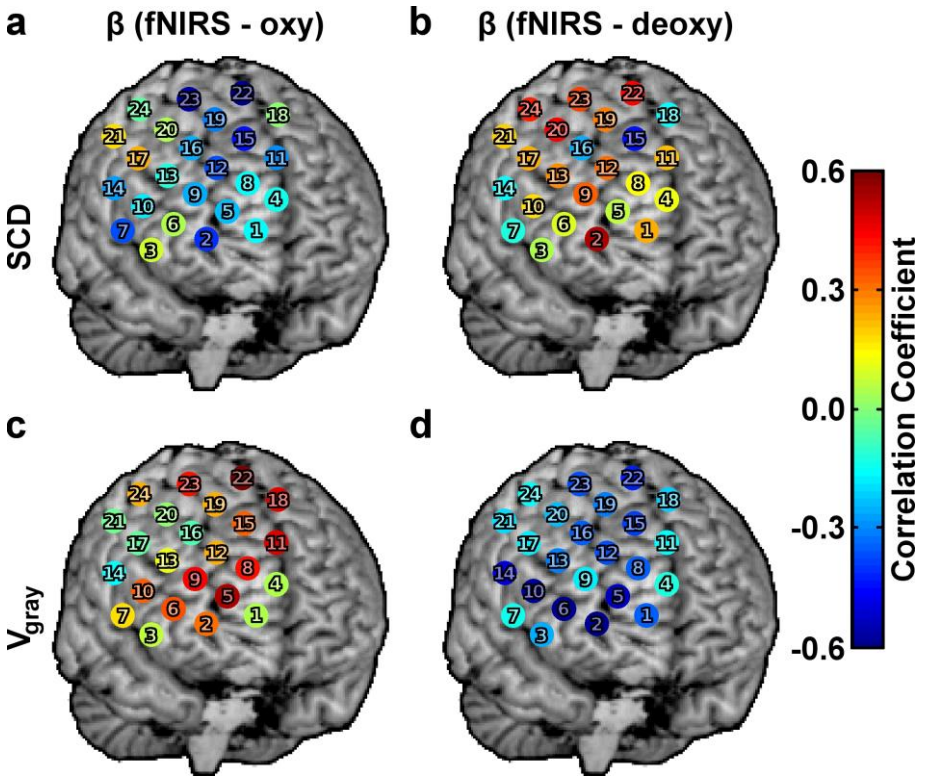

Figure 5

Impact of individual anatomy on fNIRS activation magnitude. Channel-wise correlation (Spearman) of a) fNIRS oxy activation (β-weights, "all rewards") and the scalp-to-cortex distance (SCD), b) fNIRS deoxy activation and SCD, c) fNIRS oxy activation and the channel-wise volume of gray matter (V_{gray}), d) fNIRS deoxy activation and V_{gray}.

Second, V_{gray} showed positive correlations with the activation (β-oxy, "all rewards") in channel #22 ($r = .67$, $p < .005$) as well as in #5, 11, 18 ($r > .48$, $p < .05$) (Figure 5c). The correlation coefficient (β-oxy ~ V_{gray}) range between channels was -.16 to .67 (mean r: .23 ± .22). The deoxy activation (β-deoxy, "all rewards") showed a corresponding negative correlation with V_{gray} in channel #2 ($r = -.76$, $p < .001$) and #5, 6, 10 and 14 ($r < -.49$, $p <. 05$) as well as marginally significant correlation in #22 ($r = -.42$, $p <.1$). The correlation coefficient (β-deoxy ~ V_{gray}) range between channels was -.12 to -.76 (mean r: -.33 ± .17; Figure 5d).

Functional activation and trait sensitivity to reward

The consistent group level fNIRS and fMRI activation elicited by ITC was correlated with trait SR scores (1) to investigate the link between trait SR and "immediate" and "delayed" reward processing in the right prefrontal function, (2) to further validate the comparability of fNIRS and fMRI and (3) to identify potential confounding factors affecting trait ~ fNIRS correlations.

(1) For fMRI analyses we defined a functional ROI from the cluster of activation (β-weights for "all rewards", whole brain FWE-corrected, $p < .01$) elicited by ITC in the right inferior and middle frontal gyrus. The ROI contained 132 voxels where we performed voxel-wise correlations between the SR score and the activation (β-weights) elicited by the "immediate" and "delayed" reward condition, respectively. We identified a significant cluster of 41 voxels showing a negative correlation ($r = -.54$, $p = .047$; corrected) between the SR score and activation elicited by the "immediate" reward condition (Figure 6). While for the "delayed" condition we found no significant cluster showing correlations with SR scores ($p > .2$, corrected), the mean correlation coefficient in the cluster (SR ~ "immediate"; 41 voxels) was also trendwise negative for the "delayed" reward activation ($r = -.39$, $p =.09$, uncorrected).

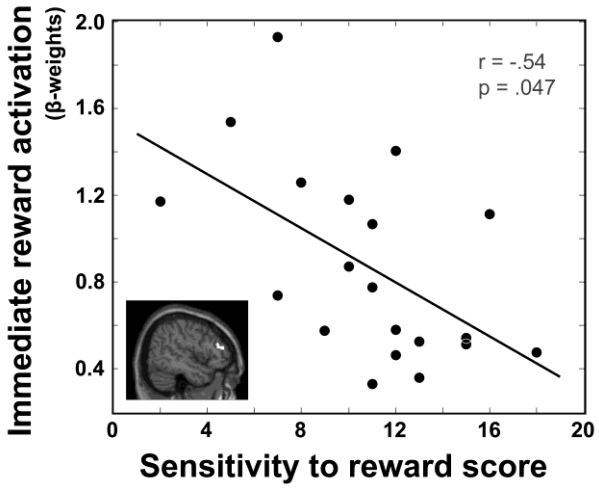

Figure 6

Scatterplot of fMRI activation (β-weights) elicited by the "immediate" rewards condition of the intertemporal choice task within a cluster (right inferior and middle frontal gyrus) showing a significant correlation (Pearson) with trait "sensitivity to reward".

(2) The fNIRS ROI contained all significantly activated channels (β oxy, "all rewards", Bonferroni-corrected; channel #2, 6, 9, 10, 13, 17, 20, 24). For the "immediate" reward condition none of the ROI channel activations showed a significant correlation with the SR score (peak $r = .33$, $p > .1$; #10). For the "delayed" reward condition channel #20 showed a positive correlation between the activation level and SR score ($r = .48$, $p < .05$).

For the deoxy activation neither "immediate" nor "delayed" reward conditions showed a significant correlation with SR scores in the ROI channels ($p > .1$). Trait SR was not significantly correlated with behavioral measures of the ITC such as decision time or the frequency of choices of the earlier reward option ($p > .1$).

(3) We investigated individual factors which might contribute to the differences in the impact of the SR score on fNIRS and fMRI functional hemodynamics, respectively. Firstly, we quantified the spatial distance of the fMRI voxel-cluster ("immediate" ~ SR score) to the closest fNIRS channels (#6, 10 and 13). The minimal distance from this cluster to the normalized fNIRS channels positions on the scalp (equidistant from emitter and detector) was larger than 35 mm. Therefore, V_{gray} in native space would

not likely spatially overlap with the fMRI cluster found in the group analyses of normalized images.

Secondly, since trait SR may affect heart rate and blood flow and, thereby, scalp blood flow fluctuations during ITC, we analyzed the impact of the SR score on the temporal correlation of fNIRS ~ fMRI V_{scalp}. SR scores showed a marginally significant positive correlation ($r = .39$, $p < .1$) with the mean r-values (oxy ~ EPI V_{scalp} time series) in channel #9 (middle frontal gyrus) whereas other correlations were not significant in the ROI ($p > .1$). For the deoxy-data, SR scores were negatively correlated with the r-values (deoxy ~ EPI V_{scalp} time series) in lateral channels near the temple region #14 ($r = -.47$, $p < .05$), #17 ($r = -.41$, $p < .1$), #21 ($r = -.52$, $p < .05$) and #24 ($r = -.53$, $p < .05$).

Discussion

We observed a high consistency between fMRI and fNIRS (oxy, deoxy) group activation elicited by intertemporal choice. Both methods showed a cluster of activation in the right middle and inferior frontal gyrus for immediate as well as for delayed reward options (Figure 2a-c). We quantified the consistency between fNIRS and fMRI signals in individual gray matter voxel clusters simulated to be traversed by near-infrared light. Temporal correlations increased with the oxy and deoxy, respectively, fNIRS activation level. However, fNIRS activation and the multimodal correlations showed a high interindividual variability. In a first step, we identified sources of error variance which may contribute to the interindividual variability in fNIRS activation and multimodality correlations. (1) Fluctuations in the scalp fMRI signal showed temporal correlations with wide interindividual variability with the fNIRS oxy (mean r ~ .2, range: -.28 to .74) and the deoxy (mean r ~ -.1, range: -.47 to .36) signal time series in the temple region extending to channels labeled as inferior and middle frontal gyrus. (2) Inter-channel and interindividual anatomical differences in scalp-cortex distance (SCD) and gray matter volumes (V_{gray}) individually simulated to be reached by the near-infrared light reduced the detected fNIRS channel activation, i.e. the

fNIRS sensitivity. Both SCD and V_{gray} showed great differences between prefrontal/forehead regions and subjects (Table 1). While the correlation of these anatomical factors with oxy and deoxy fNIRS activation, respectively, was most profound in the superior frontal gyrus and near the hemispheric cleft ($|r| \sim .05$), correlations ($|r| > .04$) were also observed for the frontal pole and temple region as well as middle frontal gyrus channels. Here, the impact of anatomical factors was more pronounced for the deoxy compared to the oxy signal.

In a second step, we aimed at identifying variance in prefrontal fNIRS and fMRI intertemporal choice activation which was explained by individual trait of "sensitivity to reward" (SR). FMRI activation elicited by "immediate" rewards within right middle and inferior frontal gyrus was negatively correlated with trait SR. Contrarily, fNIRS oxy activation of the "immediate" reward condition showed no significant correlations (but positive signs) with trait SR. However, for "delayed" rewards a significant positive correlation was found in middle frontal gyrus (channel #20). The fNIRS deoxy signal showed no significant correlation with the trait.

Anatomical and (systemic) physiological factors might explain the inconsistent fNIRS and fMRI activation ~ trait associations. The limited penetration depth of fNIRS might not have been sufficient to reach the cluster of significant fMRI activation-trait correlation. Additionally, SR scores were positively correlated with fNIRS oxy ~ scalp fMRI coupling in the middle frontal gyrus and the temple region. Thus suggesting that trait SR partly explains interindividual variability in confounding systemic physiological influences affecting fNIRS activation ~ trait associations.

The present methodological and functional findings are discussed with respect to previous findings.

Extracranial signals

Systemic physiological noise and task-evoked extracranial signal changes have been repeatedly shown to impact fNIRS signals using different techniques including fMRI, laser Doppler tissue blood flow meter probes and fNIRS with short (3 - 5 mm) emit-

ter-detector distance (Gregg et al., 2010; Kirilina et al., 2012; Saager et al., 2011; Smielewski et al., 1997; Takahashi et al., 2011). These confounding effects have been reported for different scalp positions and cutaneous compartments, including the temple area which is in close vicinity to the frontal branch of the superficial temporal vein and artery as well as the temporal muscles (Sato et al., 2011). Channels of this area showed the highest time series correlations of fMRI scalp and fNIRS, however, the influence of scalp signal fluctuations was not restricted to this area as shown by weaker correlations ($r > .1$) in more dorsal regions (middle frontal gyrus). As indicated by scalp fMRI ~ fNIRS temporal correlations, the impact on the fNIRS oxy signal was stronger and spatially less restricted compared to fNIRS deoxy. Systemic physiologic artifacts as induced by hemodynamics of cutaneous draining veins of the forehead have been shown to largely affect the oxy, but not the deoxy signal (Kirilina et al., 2012), which may partly underlie the differences in the magnitude and extent of correlations of the fNIRS oxy and deoxy signals with the fMRI scalp fluctuations. Pial veins in the motor cortex area have been shown to contaminate fNIRS signals during motor tasks, however, more strongly reducing cortical contributions for the deoxy compared to the oxy signal (Gagnon et al., 2012). In the present fNIRS-fMRI study different scalp compartments such as small vessels in superficial dermal plexuses or large cutaneous arteries or draining (pial) veins were not resolved. Variability in artery and vein morphology were therefore not investigated for differential contributions to the interindividuality of fNIRS signals. Thus, while different (micro)vascular extra- and intracranial systemic physiological artifacts may contaminate fNIRS signals, the present findings show that the impact on fNIRS may be interindividually weighted as shown by scalp fMRI ~ fNIRS correlations.

The cluster of right inferior frontal gyrus fMRI activation extending to the insula was not detected using fNIRS (channel #3 and #7). Here, extracranial systemic noise might be an explanation for the difference in fNIRS and fMRI group activation near the temple region as indicated by an increased correlation of fNIRS oxy and fMRI V_{scalp} and a decreased correlation of fNIRS oxy and fMRI V_{gray}. On an individual level, systemic physiological and anatomical sources of error variance might interact. For instance, for smaller heads (e.g. in females) channel #6 or #10 (labeled as middle

and inferior frontal gyrus in the standard brain) would be shifted towards the temple region using the probe-set positioning of the present study and would, thus, be more impacted by the systemic physiological noise of the temple region compared to larger heads (e.g. in males).

Individual anatomy

SCD has previously been shown to increase fNIRS signal variance and decrease the correlation between fNIRS and fMRI signals in right prefrontal and parietal areas using a battery of different cognitive and motor tasks (Cui et al., 2011). Consistent with these findings SCD had an impact on the magnitude of fNIRS activation (β-weights of oxy and deoxy, respectively) with interregional differences. Largest negative correlations ($r < -.5$) were present in channels of long SCD (> 15 mm) in the dorso-medial part of the prefrontal probe-set. Here, long SCD might have limited the fNIRS sensitivity which might explain that the fNIRS oxy signal did not detect activation of the supplementary motor area as shown using fMRI. However, inconsistent with this interpretation the fNIRS-deoxy which has higher spatial specificity (Franceschini et al., 2003; Hirth et al., 1996) did show activation in this area. Less strongly ($r \sim -.3$) SCD negatively correlated with fNIRS channel activation in more anterior, fronto-polar and temple regions.

However, SCD only explained 52% ($r = -.72$) of the variance in V_{gray}, which was assessed by also considering individual light scattering, reflection and absorption within individual tissue compositions and their boundaries, and should therefore represent a more sensitive indicator of individual anatomy influencing fNIRS sensitivity compared to SCD (Haeussinger et al., 2011). Compared to SCD the V_{gray} showed a similar spatial distribution of impact on activation magnitude. However, in some functional ROI channels of the inferior and middle frontal gyrus (#6, 9, 10), where SCD (~ 11-13 mm) and its inter-subject variability (1-2 mm) were rather small, V_{gray} showed relatively greater impact on the activation magnitude (oxy: mean $r = .37$, deoxy: mean $r = -.47$) compared to SCD (oxy: mean $r = -.12$, deoxy: mean $r = .30$). In these infe-

rior/middle frontal gyrus channels variance of the fNIRS activation was explained by individual anatomy as assessed by V_{gray} by up to 18% for the oxy signal, and by up to 41% for the deoxy signal. Here, SCD explained up to 6% (oxy) and 20% (deoxy), respectively, of the channel activation variance. Thus, individual anatomical influences determining fNIRS sensitivity for measuring gray matter functional hemodynamics go beyond SCD, and are more complex and non-linearly related to SCD (Haeussinger et al., 2011).

For both SCD and V_{gray} the impact of individual anatomy in channels of the functional region of interest (inferior and middle frontal gyrus) was more pronounced for the deoxy compared to the oxy signal. The ITC deoxy activation cluster was spatially more restricted which corresponds to the reported better regional specificity of the deoxy signal with more venous than arterial contributions to the signal compared to oxy (Franceschini et al., 2003; Hirth et al., 1996). However, this regional specificity of deoxy might be accompanied with an increased impact of individual anatomy relative to the oxy signal.

Practically, since the mean SCD has been shown to be correlated with head circumference ($r = .46$) (Haeussinger et al., 2011), and differences in head size might affect relative channel positions, future fNIRS studies should control for head circumference to minimize anatomy-related interindividual variance.

Functional activation during intertemporal choice

Previously, for fNIRS-fMRI temporal correlations coefficients of $|r| \sim .4$ in right prefrontal fNIRS channels activated during visuo-spatial working memory, go-nogo response inhibition, or judgment of line orientation tasks have been reported (Cui et al., 2011). The relatively smaller mean correlations ($|r| \sim .2$) reported in the present study might be due to the characteristics of the ITC task. Here, the presentation, evaluation and decision-making between the two reward options might show stronger intra- and inter-region variability in emotional and cognitive neural processing compared to mainly cognitive tasks. However, emotional and cognitive processing within cortico-limbic networks has been implicated in many traits of personality and

psychopathologies, such as bipolar disorder, depression, schizophrenia and addiction (Heatherton and Wagner, 2011; Morris et al., 2012). Since fNIRS is increasingly used for cortical measurements of differences in cortico-limbic processing, we chose a functional paradigm engaging these structure, and hypothesized trait SR-related interindividual differences in cortical processing to be detected using fNIRS (and fMRI) despite interindividual anatomical and physiological sources of error variance.

Since analyses were focused on fNIRS-fMRI (functional) hemodynamic responses within the right prefrontal cortex and its role for cognitive control, analyses of activation within subcortical structures involved in subjective reward valuation and reward choices was not in the scope of the present study.

The fMRI finding of a negative impact of trait SR on inferior/middle frontal gyrus activation during the presentation of "immediate" rewards is consistent with the role of these structures for cognitive control. Interestingly, the correlation with "delayed" reward activation was not significant (but also negative) indicating that trait SR has a differential impact depending on reward availability. Consistent with this trait-activation coupling for immediate rewards, SR scores were significantly positively correlated with the self-rating of how urgent a subject would need 20 € ($r = .45$, $p < .05$), but not with the self-rating of the general momentary financial situation ($p > .1$). However, trait SR was not significantly correlated with ITC decision time or choice behavior ($p > .1$) indicating that the impulsivity reflected by the construct of trait SR might not be directly related to impulsive behavioral measures of ITC.

Neural task-related fNIRS signal variance in inferior and middle frontal gyrus could not be explained by trait SR as shown using fMRI; possibly due to aforementioned sources of fNIRS error variance which even might have biased correlations in the positive direction. Some indication for such a bias is given by the increased correlation between fNIRS ~ scalp fMRI in the temple and middle frontal gyrus region with increasing SR scores, and the distance between fNIRS channels and the fMRI voxel cluster showing the correlation. However, it is unclear from our scalp fMRI recordings what exact systemic physiological impact increased SR might have on systemic physiological responses during the ITC and the possibly decreased fNIRS sensitivity for cortical activation. The heart rate and blood pressure might be increased throughout

the task, particularly during the anticipation of the next trial reward option, or at the moment of their presentation.

Limitations of the study

The fMRI EPI signal has low signal intensity in the scalp and might therefore lack sensitivity for measuring individual and task-evoked changes in scalp hemodynamics. Also, resolving contributions of arterial, venous or skin compartments is not possible with an EPI-voxel size of 3.3 mm × 3.3 mm × 4.0 mm (at 1.5 T) which may only give a rough estimate of scalp hemodynamics and its impact on fNIRS measurements.

Conclusions

For fNIRS group analyses aiming to quantify functional task-related brain activation and, in particular, the investigation of individual factors impacting neural activation, multiple and individually weighted sources of physiological and anatomical error variance might have to be considered. For different task types and fNIRS channel scalp positions these potentially confounding effects should be quantified and controlled for in healthy and (psycho)pathological populations. Technical (e.g. multi-distance optode recordings and supplementary physiological and morphometric measurements) and analytical advancements (e.g. independent component analysis) should further address the issue of individuality to improve the validity of optical neuroimaging.

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General discussion

The present cumulative dissertation comprises three human neuroimaging studies investigating hemodynamic or electrical correlates of prefrontal cortex (PFC) function as well as methodological aspects of functional near-infrared spectroscopy (fNIRS). Using different neuroimaging techniques, functional paradigms and diverse individual factors of molecular genetics, (neuro)anatomy and morphology, demographic factors (e.g. age, sex, years of education), behavior, personality and psychopathology, specific scientific hypotheses regarding prefrontal task-specific activation were addressed. The findings, implications and future prospects of each study are critically discussed in a general context and in the context of recent evidence and theories.

Study #1:

"*COMT*×*DRD4* Epistasis Impacts Prefrontal Cortex Function Underlying Response Control"

The study investigated the impact of two dopaminergic gene variations and their interaction on neural and behavioral correlates of prefrontal cognitive responses control using EEG and a Go-NoGo task in a large sample of 114 healthy controls and 181 adult patients with ADHD. The study showed that an epistastic interaction between *COMT*-dependent prefrontal dopamine levels and *DRD4*-dependent inhibitory D4 receptor function (*DRD4*) impacts on neural and behavioral measures of prefrontal cognitive response control.

The key hypothesis underlying this epistatic interaction is that both, (*COMT*-dependent) dopamine levels as well as differential dopamine receptor stimulation (excitatory D1 versus inhibitory D2/D4 stimulation ratio), may critically modulate prefrontal processing through an imbalance in dopamine-mediated excitation and inhibition within glutamatergic and GABAergic neural networks. Here, more stable (D1-dominated state) or more flexible (D2/D4-dominated state) neural states (Durstewitz and Seamans, 2008) may be optimal for neural and behavioral outcomes depending on the nature of a given task situation (Cools and D'Esposito, 2011). Here, the present findings suggest that subjects with intermediate dopamine-levels (associated

with stable, D1-dominated processing) have an increased risk of impaired flexibility when D4-receptor function is additionally decreased. These subjects showed reduced neural flexibility such as less efficient transitions from neural Go to NoGo representations (decreased NoGo-anteriorization) limiting performance as indicated by increased "Go" response reaction times and its intraindividual variability. Response inhibition centrally involves the anterior cingulate cortex as well as inferior frontal gyrus and cortico-thalamic motor circuits (Aron and Poldrack, 2006; Fallgatter et al., 2002). Here, exploratory source localization analyses indicated that the behavioral consequences of the epistatic effect might emerge from the level of the right premotor and supplementary motor area. However, the precise cellular effects and neural (network) dynamics which may be affected by *COMT*×*DRD4* epistasis were not investigated in this Imaging Genetics study. Specifically, the (genetic) dopaminergic modulation of the neural interaction between prefrontal excitatory glutamatergic pyramidal neurons and GABAergic interneurons was not investigated. Since ADHD patients did not significantly differ from healthy controls in regard to *COMT*×*DRD4* epistasis, the precise mechanism of this dopaminergic (gene) interaction might not be centrally involved in the pathophysiology of ADHD. However, ADHD patients showed lower P300 amplitudes at Cz and Pz positions, where the neural dynamic flexibility of P300 amplitudes in the transition from Go to NoGo trials was less pronounced compared to healthy controls. These findings may represent an endophenotype of the pathophysiology underlying deficits in response inhibition in ADHD, and it will be important to investigate the genetic and cellular basis of the altered neural processing in future studies.

In the following, further implications and possible frameworks and hypotheses for future studies derived from the present findings are given.

In addition to ADHD, dopaminergic dysregulation and deficits in prefrontal executive functions are also common for other clinical conditions such as schizophrenia (Barch and Ceaser, 2012), addiction (Koob and Volkow, 2010) or Parkinson's disease (Ko et al., 2012). Tolcapone, a pharmacological centrally and peripherally acting COMT inhibitor, has been reported to improve motor functions in Parkinson's disease (Bonifacio

et al., 2007) and might improve cognitive and negative symptoms of schizophrenia and impulsivity in addiction. Currently, several clinical trials investigate the effects of tolcapone treatment on these symptoms (search "tolcapone" at: www.ClinicalTrials.gov). Importantly, these studies specifically investigated the effect of the individual *COMT* Val158Met genotype on treatment outcome for the purpose of a personalized, genotype-informed medication in the future.

In healthy subjects tolcapone has repeatedly been shown to enhance working memory performance and increase DLPFC working memory brain action in dependence of *COMT* Val158Met genotype (Apud et al., 2007; Farrell et al., 2012). COMT inhibition improved performance and increased DLPFC activation in subjects with relatively low dopamine levels and COMT enzymatic function degrading dopamine (Val/Val genotype), whereas the opposite pharmacological effect was observed in COMT Met/Met carriers which, without medication, have relatively increased dopamine levels and activation, and better working memory performance (Apud et al., 2007; Farrell et al., 2012). Thus, patients and healthy subjects carrying the Val-alleles might benefit from pharmacological COMT inhibition while the opposite might be the case for Met/Met carriers.

Working memory centrally involves a neural stable online representation. The findings of the present study suggest that for tasks involving such stable neural processing Val-carriers might benefit from pharmacological COMT inhibition, however, subjects with additionally decreased inhibitory D4-receptor function might exhibit overly decreased neural and behavioral flexibility. Thus, in addition to *COMT* Val158Met, genotypes affecting dopamine receptor function should be considered to optimize pharmacological effects in respect to (executive) functions important for well-being and daily living. Conversely, antipsychotic treatment in schizophrenia and bipolar disorders using (non-selective) dopamine receptor (D2 type) antagonists should be further investigated for an impact of COMT genotype. A moderating role of *COMT* genotype for the effect of antipsychotic medication on different executive functions in patients with schizophrenia have been reported showing better symptom improvement for Met-allele carriers compared to Val-carriers (Bertolino et al., 2004; Woodward et al., 2007).

In addition to *COMT* and *DRD4*, many other genetic factors may sculpt the dynamics of PFC processing, the regulation of neural excitation and inhibition and ultimately behavioral and clinical phenotypes. For future Imaging Genetics and genetic association studies the present findings may provide important hypotheses. First, the study emphasizes that phenotypic differences in PFC processing dynamics and behavior might emerge through genetic epistasis while main effects of single genes may not be detected. Second, *COMT* Val158Met is possibly the most studied SNP in (psychiatric) neuroscience. However, especially in tasks or traits for which stable versus flexible processing or behavior is required, inconsistencies in findings or additional variance especially in Val/Met carriers could be explained by genetic factors modulating dopamine receptor function, such as the *DRD4* 48-bp VNTR polymorphism. Moreover, studies stratifying for *COMT* homozygotes miss genetic and functional variability in heterozygotes which might be due to interactions with *DRD4* genotype. Since heterozygotes account for about 50% of the Caucasian population, the generalizability of the impact of *COMT* genotype on neural and behavioral phenotypes might be limited in these studies. Third, biological pathways and mechanisms are highly important to select genetic variants and generate hypotheses of main and interaction effects on different phenotypic markers within the brain. Dopamine levels as well as the ratio of excitatory versus inhibitory dopamine receptor stimulation modulating glutamatergic and GABAergic neurons and stable and flexible neural states may provide hypotheses for genetic interactions such as *COMT* × *DRD4.* Imaging Genetics and genome wide association studies (GWAS) might benefit from such *a priori* hypotheses because the access to individual genetic information has grown immensely. Nowadays, some studies investigate over 500,000 SNPs for each subject and, thus, face severe multiple testing problems, especially when epistatic interactions and numerous dependent variables, e.g. 50,000 voxels in fMRI, are included in the statistical analyses (Stein et al., 2010). Complementing such rather exploratory studies, the interpretation and biological or pathophysiological relevance of significant findings might sometimes be more conclusive when following biologically and clinically informed hypotheses derived from previous findings.

Study #2

"Aging-related cortical reorganization of verbal fluency processing:
a functional near-infrared spectroscopy study"

Increased age is the greatest risk factor for neurodegenerative diseases, such as Alzheimer's disease (AD), and deficits in verbal fluency represent one of the earliest cognitive symptoms of AD. Moreover, pathological alterations on the molecular, cellular, neural network and vascular level precede the first cognitive symptoms by years or even decades (Jack et al., 2010).

Therefore, the predictive value of age as well as sex, verbal fluency performance and years of formal education of cortical functional hemodynamic responses elicited by phonological and semantic verbal fluency were investigated in the present fNIRS study. In total, data of 325 elderly, non-demented subjects between 51 and 82 years of age was analyzed as part of the fNIRS baseline assessment of the longitudinal TREND study. Aim of this study is to identify risk factors of Alzheimer's disease and Parkinson's disease, respectively, for an early detection of these neurodegenerative diseases in subjects who are still symptomatically healthy.

The investigated predictors of the cortical activation elicited by the task showed small effect sizes ($-0.24 < \beta < 0.22$) while the multiple regression models comprising all predictors explained up to 10% of the variance of fNIRS data. Age significantly predicted both, decreased bilateral inferior frontal junction (IFJ) responses and increased bilateral middle frontal gyri and supramarginal gyri responses. This reorganization of activation with increasing age might indicate an aging-related neural compensation strategy. This finding is discussed in regard to effects of the other investigated predictors and the potential of the present findings to aid an early prediction of mild cognitive impairment and AD.

Increased activation in middle frontal gyri and supramarginal gyri could indicate increased involvement of cognitive control functions (Bush and Shin, 2006; Niendam et al., 2012). Executive functions important for verbal fluency performance, such as flexibility, inhibition, initiation, and working memory as well as attention processes have been shown to activate fronto-cingulo-parietal networks comprising the pre-

frontal and parietal regions found to be more strongly activated with increasing age in the present study.

The effortful mental search for lexical representations and verbal fluency performance with increasing age might be ameliorated by increased neural recruitment subserving these executive functions, and thereby compensate potential aging or first neurodegeneration-related deficits on multiple organization levels of the brain. Some indication of such neural decline might be represented by the decrease in bilateral IFJ (functional) hemodynamic responses with increasing age. Supporting this interpretation, glucose hypometabolism assessed using positron-emission tomography in early dementia patients correlates with executive deficits of semantic fluency ("Name items in a supermarket!") in the left IFJ (Schroeter et al., 2012). However, for aging-related bilateral functional hemodynamics in the IFJ/temple area measured using fNIRS cautious interpretations of the hemodynamics as neural activation are advised; see below for critical discussion of methodological aspects of fNIRS.

Neural and behavioral processes involved in verbal fluency and cognitive/attention network activity might also be modulated by other factors partly confounded with aging. For instance, (1) depressive symptoms, (2) alertness/fatigue, (3) other motivational and psychological aspects or (4) articulatory movements during speech production (Rusz et al., 2011) and psychomotor speed (Hipp et al., 2009), e.g. in subjects with early Parkinson's disease. In part, these additional factors may be correlated with age while not being directly associated with neurodegenerative processes involved in AD.

(1) Depression has repeatedly been associated with bilateral prefrontal hypoactivation during verbal fluency without concomitant performance deficits (Klumpp and Deldin, 2010). Depression also represents a risk factor for Alzheimer’s disease (Ownby et al., 2006), however, variance in fNIRS data due to depressive symptoms was not in the scope of the present fNIRS study. The Alzheimer’s disease risk factor of depression is investigated in the TREND study and will be examined in further fNIRS analyses. (2) In the TREND study subjects participate in eight different twenty-five minute experiments including neuropsychological testing (CERAD-Plus battery) or motor and neurological tests, which were completed in random order within ap-

proximately three and a half hours. For some older participants the successive experiments might have been more challenging, and fatigue might have decreased performance or increased (neural) efforts to sustain attention and executive functions. Interestingly, analyses on this issue showed that the position of fNIRS within the order of the eight experiments, showed a small positive correlation ($r \sim .1$) with the phonological and semantic verbal fluency performance. While no longer significant, these correlations had a positive sign in groups of individuals over and under an age of 65 years, respectively. Therefore, participants tended to perform better after participation in other experiments, possibly because the experimental testing situation became more familiar. (3) Older subjects or subjects with a family history of AD may have been especially motivated to perform well in the verbal fluency task to disprove a personal risk of cognitive impairment. In turn, resignation or fatalistic attitudes might produce opposite effects. Thereby, increased/decreased activation of cognitive/attention fronto-cingulo-parietal networks might be psychologically explained. (4) Early Parkinson's disease can be accompanied with symptoms of altered vocal phonation and articulation (Rusz et al., 2011) as well as psychomotor speed (Hipp et al., 2009) which might impair verbal fluency or require increased neural resources underlying executive functions and attention. While these motor-related deficits differ from declining cognitive functions of effortful word retrieval, both might be correlated with increasing age.

Thus, multiple other psychological and pathological factors might be correlated with age and might be confounded with aging processes and their neural and behavioral correlates of verbal fluency. In addition to age, other variables significantly predicted (with small effect sizes) the cortical activation measured using fNIRS. Cognitive performance correlates the level of education for all ages, which may in part underlie the increased risk factor of dementia in individuals with low educational attainment levels (Caamano-Isorna et al., 2006). Consistent with these findings, years of education positively correlated with phonological ($r = .39$, $p < .001$) and semantic ($r = .15$, $p < .01$) verbal fluency performance, whereas age was only weakly correlated with semantic ($r = -.13$, $p < .05$), and did not correlate phonological verbal fluency performance.

Moreover, while no correlation between age and years of education was found, the two predictors showed partly opposing effects on the neural level. Longer education was a predictor for higher phonological verbal fluency activation in bilateral IFJ and decreased left middle frontal gyrus activation. No significant link between the cortical activation and verbal fluency performance was found suggesting that the neural effects of education might not exclusively be due to better performance. The neural differences might indicate that, for instance, (1) different cognitive strategies during effortful search for words matching the task condition criteria were employed in more educated individuals, e.g. more flexible switching between categories of syntactically connected words than clustering of words in one category, thereby generating more words (Troyer et al., 1997). (2) In addition to different behavioral strategies, more educated subjects with possibly increased crystallized and fluid intelligence might have a higher cognitive reserve, i.e. neural efficiency, capacity or compensatory recruitment of additional brain regions (Stern, 2006; Tucker and Stern, 2011). These neural mechanisms might underlie the present differences of the impact of age and education on the regional neural activation.

Also, higher education reflecting cognitive capability might reduce the risk for MCI and AD or delay their symptomatic effects (Hall et al., 2007; Sharp and Gatz, 2011), but a steeper decline in cognitive functions has been shown for more educated subjects when first AD symptoms occur (Bruandet et al., 2008; Scarmeas et al., 2006). Thus, education may represent an important additional predictor of neural and behavioral functions underlying aging and neurodegenerative processes. However, education might be associated with potential confounders such as socioeconomic status and life-style, and these factors should be taken into account in future analyses of the TREND study, wherein the average education of 14.3 ± 2.9 years (mean ± SD, n = 325) indicated a high educational level and participation of many academics.

Females showed higher activation within bilateral IFJ, but lower bilateral middle frontal gyri and supramarginal gyri responses compared to males. In part these sex-differences in regional activation might reflect performance differences (females on average slightly outperformed males) related to task performance strategies such as increased switching between word-categories in females compared to males (Lanting

et al., 2009). The neural differences in females compared to males were similar compared to the effects of longer education although females had significantly shorter education (on average 1.3 years less). In this regard, the left inferior frontal gyrus has been shown to be more strongly activated during switching compared to free generation in semantic verbal fluency (Hirshorn and Thompson-Schill, 2006) suggesting that neural differences between males/females, or due to education level, might be related to differences in behavioral strategies. Moreover, the differences in behavioral strategies and underlying neural processing might also partly explain the lack of significant task performance effects on regional activation magnitudes.
Further analysis of the fNIRS data in an extended sample of 654 subjects confirmed the present significant predictors with no major changes in effect sizes (Heinzel et al., 2012). Additionally, increased verbal fluency performance predicted increased left IFJ ($\beta = .09$, $p < .05$) activation in the larger sample consistent with its role for behavioral switching strategies improving performance (Hirshorn and Thompson-Schill, 2006).

For a critical general discussion of the present fNIRS findings a distinction between two possible sources of fNIRS data variability, the previously discussed actual neural and cortical functional hemodynamics on the one hand, and (1) vascular factors and (2) possible task-evoked systemic physiological artifacts on the other hand, might be appropriate.
(1) Functional hemodynamic responses may represent neural correlates of brain functions as neurovascular coupling via astrocytes reliably mediates local increases in cerebral blood volume, flow and oxygenation following neuronal (glutamatergic) synaptic activity (Haydon and Carmignoto, 2006; Iadecola and Nedergaard, 2007; Logothetis, 2002). Thus, compromised vascular function and neurovascular coupling might hinder a straight-forward interpretation of hemodynamic signals as neural activity. For aging as well as hypertension, stroke and AD alterations in hemodynamic responses have been reported (D'Esposito et al., 2003; Girouard and Iadecola, 2006). Vascular risk factors, such as hypertension, diabetes mellitus, cerebrovascular diseases, and hypercholesterolemia increase the AD risk (Barnes and Yaffe, 2011;

Murray et al., 2011). Moreover, cerebral amyloid angiopathy, i.e. the accumulation of amyloid beta-peptides on cerebral blood vessels, is associated with cerebral hypoperfusion and cognitive decline and represents one of the hallmarks of AD (Bell and Zlokovic, 2009). Moreover, smoking, obesity and pharmacological medication may affect vascular function or neurovascular coupling. For instance, aspirin (acetylsalicylic acid) has antipyretic, anti-inflammatory, analgesic and anti-coagulant effects and is often prescribed as long-term low dose medication to prevent blood clot, strokes and heart attacks (Lewis et al., 1983). Aspirin is an irreversible inhibitor of cyclooxygenase (COX-1, COX-2) involved in the astrocyte-mediated neurovascular coupling which is affected by genetic (Hahn et al., 2011) and pharmacological factors inhibiting COX enzymatic function (Bruhn et al., 2001). Due to regular aspirin intake 13% of the participating subjects were excluded from fNIRS analyses. Since vascular pathologies such as atherosclerosis, coronary heart disease or hypertension have a high incidence in subjects over 50 years of age, future fNIRS analyses will address the issue of altered hemodynamics due to these pathologies or medication using aspirin, antihypertensive drugs and other frequent drugs in the TREND cohort.

(2) Participants of the TREND study may perceive the verbal fluency test as a stressful experimental situation. An example of a verbal instruction during the fNIRS measurements is: "List as many nouns starting with the letter F as you can! (You have 30 seconds.)" First, the task, e.g. generating words on the basis of orthographic criteria, is largely unfamiliar to many participants and unusual in regard to daily cognitive activities. Optimal verbal fluency performance requires efficient organization of verbal retrieval, verbal recall and cognitive self-monitoring, effortful self-initiation, and inhibition of responses as well as other executive functions. While the task may be well suited to identify cognitive deficits (Henry and Crawford, 2004; Henry et al., 2004), its performance may increase stress levels and arousal. Second, subjects (intentionally) participate in a study which might reveal a personal risk or first symptoms of mild cognitive impairment or AD. Accordingly, performing a cognitively demanding task such as the verbal fluency test while brain processes are being monitored may induce additional stress or psychologically challenge subjects, especially when performance is perceived as poor.

Importantly, task-evoked systemic physiological hemodynamic changes, such as changes in heart rate and blood pressure, elicit intra- and extracranial hemodynamic changes in different micro- and macrovascular compartments and, thereby, contaminate fNIRS signals (Kirilina et al., 2012; Minati et al., 2011; Takahashi et al., 2011). Changes in the concentration of oxygenated and deoxygenated hemoglobin, respectively, in the extracortical vascular compartments traversed by near-infrared light affect light intensity at the light detector, confounding functional hemodynamic responses taken to reflect brain activation in cortical gray matter. For the temple region containing the extracranial frontal branches of the temporal artery and vein, fNIRS signal changes have been shown to underlie these extracranial and not cortical hemodynamics (Sato et al., 2011) (see also study #3 below). The (functional) hemodynamic responses and their correlation with age, gender and years of education in the temple region/IFJ should therefore be interpreted with caution.
Verbal fluency task conditions might not only differ in cognitive demands but also in stress levels and arousal, which may contribute to differences in (functional) hemodynamic response magnitudes between task conditions, e.g. "Name as many flowers as you can!" versus "Recite the weekdays!" (semantic fluency condition versus control task).

To conclude, currently fNIRS as used in the present study cannot dissociate between hemodynamic signal changes due to neural processing and variance in this signal due to (aging-related) vascular factors or task-evoked systemic physiological artifacts. For valid interpretations of fNIRS signals on a single subject level and fNIRS as a reliable tool for the personal early prediction of cognitive decline and neurodegeneration, the following issue have to be addressed. The analysis of individual fNIRS data would have to account for (1) vascular (risk) factors affecting (functional) hemodynamic responses, (2) systemic physiological influences on fNIRS signals, (3) individual head morphology and (neuro)anatomy (see study #3 below), and (4) individual (macro-)vascular morphology.
However, vascular risk underlying cerebral hypoperfusion and neural correlates of cognitive processing may in combination represent promising predictors of MCI and

AD. The research on vascular and neural risk of AD will benefit from further fNIRS studies, such as conducted as part of the TREND study, where fNIRS data sets of over 1100 participants have been recorded in only about four months. In the near future, these fNIRS data sets are analyzed by also considering genetic data, blood-based biomarkers, vascular risk profiles, medication, neuropsychological tests, medical histories and other measures, and in regard to longitudinal comparisons of the data of each individual participant.

Study #3

"Variability of (functional) hemodynamics as measured with simultaneous fNIRS and fMRI"

The present study first aimed to compare prefrontal fNIRS and fMRI group activation patterns and quantify individual functional hemodynamic responses elicited by intertemporal choice. Second, fNIRS activation magnitudes were investigated for an impact of individual anatomy, i.e. channel-wise scalp-cortex distance and cortical gray matter volume (V_{gray}) simulated to be reached by the near-infrared light. Third, systemic physiological artifacts affecting fNIRS signals were investigated by correlating the fNIRS time series with fMRI signal fluctuations within the scalp. Fourth, prefrontal fNIRS and fMRI activation during intertemporal choice was investigated for consistency in regard to correlations with trait sensitivity to reward (SR), and anatomical and systemic physiological artifacts affecting fNIRS activation-trait associations.
On the group level (20 healthy subjects) the cluster in the right inferior/middle frontal gyrus activation was consistently found by the two simultaneous but independent neuroimaging methods and by previous fNIRS and fMRI studies (McClure et al., 2004; Plichta, 2009). However, fNIRS did not detect additional activation in right inferior frontal gyrus/insula in the temple region as shown by fMRI.
Temporal correlations of the two methods showed wide interindividual and interregional variability. For instance, in the fNIRS peak activation channel (oxy signal, channel #13, middle frontal gyrus) the temporal correlation between the entire time-series of the fNIRS signal and the corresponding fMRI signal in cortical gray matter

simulated to be reached by the near-infrared light, varied from r = -.01 to r = .62 between subjects (mean ± SD; r = .18 ± .17). Additional analyses showed that this interindividual variability was also present for temporal correlations of the event-related average segments of fNIRS and fMRI signals with a mean correlation of r = .57 ± .42 (range: -.76 to .95). A similar degree of interindividual variability in such temporal multimodal correlations has previously been reported for a task battery joint analysis of channel-wise fNIRS-fMRI correlations during motor, response inhibition, visuo-spatial orientation judgment and working memory tasks in right parietal and right prefrontal regions (Cui et al., 2011).

Generally, variance in fNIRS and fMRI activation, respectively, and their multimodal correlations, may be largely determined by interindividual variance in neural task processing and by error variance. For fNIRS, major sources of error variance are given by individual anatomy (Cui et al., 2011; Haeussinger et al., 2011) and by systemic physiological artifacts affecting fNIRS signals and sensitivity for measuring neural activation (Kirilina et al., 2012; Minati et al., 2011; Sato et al., 2011; Takahashi et al., 2011). Individual heads differ in size and shape, but also in the thickness of different layers of skin/muscle tissue (scalp), skull, air (in the frontal sinus region), cerebrospinal fluid, and cortical gray matter. We previously showed that in addition to the scalp-cortex distance (SCD) also the individual composition of these tissue layers varies between subjects and prefrontal/forehead regions (Haeussinger et al., 2011). Moreover, using Monte-Carlo simulations we showed that these anatomical differences are accompanied with differences in the volume of gray matter reached by near-infrared light. The present study showed that these anatomical measures impact the magnitude of fNIRS functional hemodynamic responses, i.e. sensitivity of fNIRS for measuring neural correlates of PFC function.

Thus, on a single subject as well as on the group level activation variance can be partly explained by individual anatomical measures. Complementing previous findings (Cui et al., 2011), SCD and V_{gray} showed the greatest impact in the dorso-medial part of the forehead (superior frontal gyrus) with longest SCD and smallest V_{gray} values. Here, the fNIRS activation showed correlations of $|r| > .05$ with these anatomical

measures. Therefore, fNIRS studies investigating neural functions in this region, e.g. task-switching in (pre-)supplementary motor cortex (Cutini et al., 2008) should account for the impact of anatomy especially when investigating interindividual or group (male versus females) differences. However, while more pronounced compared to SCD the more specific anatomical measure of V_{gray} showed correlations with fNIRS activation of channels within the inferior/middle frontal gyrus channels where up to 18% of the oxy and up to 41% of the deoxy fNIRS activation variance was explained by V_{gray}. Thus, individual anatomy also plays a role for fNIRS sensitivity in inferior/middle frontal gyrus or (dorso)lateral prefrontal cortex where neural correlates of cognitive functions in healthy subjects, e.g. (Cazzell et al., 2012; Ernst et al., 2011), and psychiatric patients, e.g. (Ehlis et al., 2008; Jourdan Moser et al., 2009), are commonly investigated using fNIRS.

Regarding the impact of (task-evoked) arousal as assessed by fMRI scalp signal fluctuations, pronounced interindividual differences were shown. Positive correlations between fNIRS and scalp fMRI time series were primarily found in the temple region although extending to channels labeled as middle frontal gyrus. In the temple region correlations of up to r = .74 (r = .20 ± .28; mean ± SD between subjects) for the oxy and corresponding negative coefficients for the deoxy fNIRS signal of up to r = -.71 (-.08 ± .30) were found. The fNIRS peak activation channel in the right middle frontal gyrus also showed wide variability between subjects with fMRI scalp signal correlations of up to r = .73 (r = .16 ± .19) for the oxy and r = -.50 (.04 ± .22) for the deoxy fNIRS signal.

While the impact may differ between subjects, systemic physiological artifacts contaminating fNIRS signals are most pronounced within, but not restricted to the temple region. Functional tasks known to elicit changes in heart rate and blood pressure due to (emotional) arousal should be cautiously interpreted, especially when correlating individual factors which might be confounded with these physiological responses (see study #2 and below) or when comparing groups with differences in emotional and/or systemic physiological responses, such as healthy controls versus panic disorder patients or vascular dementia patients. In addition to the implications for future fNIRS task designs, advances in the fNIRS technology and analysis methods currently

address this issue. For instance, fNIRS optodes of multiple distances can enable monitoring and correction of fNIRS data (recorded at 3 cm interoptode distance) using a regressor for hemodynamic signals measured in the scalp (e.g. at 5 mm interoptode distance) (Funane et al., 2012; Gagnon et al., 2012; Saager and Berger, 2008; Takahashi et al., 2011).
Moreover, principal or independent component analyses (PCA, ICA) may be used to decompose a set of different signal components to partly identify and remove signal contribution from extracerebral tissue (Kirkpatrick et al., 1998; Virtanen et al., 2009).

In addition to the present findings of individually weighted and region dependent sources of error variance in fNIRS data, variability differences in neural processing may also underlie differences in cortical functional hemodynamic responses. The present study used an intertemporal choices task as functional paradigm since interindividual differences in neural processing were hypothesized (Peters and Buchel, 2011). Specifically, inferior and middle frontal gyrus have been shown to be involved in cognitive control processes mediating the top-down regulation of the motivational system comprising limbic and other subcortical structures. In this regard the personality construct of trait sensitivity to reward was hypothesized to be associated with a decrease in the activation in inferior/middle frontal gyrus underlying cognitive control. Using fMRI we confirmed this hypothesis as during the evaluation and choice of reward options also involving immediate rewards the activation in this cortical region showed a negative correlation with the trait. When both reward options of choice were only available after a delay of a minimum two weeks the correlation was not significant. However, using fNIRS no association between the trait and cortical activation was detected. Thus, the previously identified individually weighted sources of error variance of fNIRS data present in inferior/middle frontal gyrus might have affected or biased the trait-activation association which exhibited a non-significant positive correlation. While the detection of this association might not have been robust enough against anatomical error variance (V_{gray}) or the distance to the correlation voxel cluster identified with fMRI, systemic physiological artifacts might have biased the negative correlation shown with fMRI in the positive direction. Some evidence for

such a confounding association between trait sensitivity to reward with systemic physiological effects during the reward-based decision making task was revealed by positive correlations between the trait and the time series correlations between fNIRS and scalp fMRI signal fluctuations. Thus, in some subjects with high trait SR scores extracerebral hemodynamics might have produced an increase in fNIRS signals although, as suggested by the fMRI findings, the functional hemodynamic response due to neural processing would be relatively decreased compared to subjects with low trait SR and putatively increased cognitive control functions during intertemporal choice.

The present study focused on right prefrontal activation during intertemporal choice and an impact of trait SR on the processing of subcortical structures or cortico-limbic interactions was therefore beyond the scope of the present study. However, the missing link between trait SR and behavioral consequences in intertemporal choice behavior or decision times might be explained by further analyses of the ventral striatum, posterior cingulate cortex and medial prefrontal cortex, which have been postulated as a valuation network involved in intertemporal choice (Kable and Glimcher, 2007). Thus, behavioral consequences of the trait SR modulation of cognitive control via right inferior/middle frontal gyrus engagement might emerge from the interaction of this prefrontal region with the subcortical valuation network. Thereby, the personality construct of trait SR could be implemented into distributed and interacting neural networks, which underlie reward valuation, prospection and decision-making involved in intertemporal choice (Peters and Buchel, 2011).

Concluding remarks

As concluding remarks of this cumulative dissertation I would like to point out the highly innovative methods, interdisciplinary approaches and the increasingly growing knowledge in current neuroscience. While largely unforeseeable in its specific nature, major advances and discoveries in the next decade(s) can be expected, which may partly be due to a major paradigm shift in neuroscience: The immense computational

and methodological advances and the massive increase in access to genetic, cellular, biomarker, structural and functional neuroimaging, behavioral, demographic and epidemiological data in animal models and humans enable research and innovative ideas which may be quantified by the publication of over 60,000 neuroscientific papers per year (Waldrop, 2012).

Future developments in information technologies might help to link new findings to current knowledge, generate new testable hypotheses, and integrate new findings into a (simulated) working model of the human brain and brain-related diseases (Markram, 2012).

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List of scientific publications

Publications as First Author *equal contribution; Impact factor (IF**)**

1. **Heinzel, S.**, Dresler, T., Baehne, C.G., Heine, M., Boreatti-Hummer, A., Jacob, C.P., Renner, T.J., Reif, A., Lesch, K.P., Fallgatter, A.J.*, Ehlis, A.C.*, 2012. COMT x DRD4 Epistasis Impacts Prefrontal Cortex Function Underlying Response Control. *Cerebral Cortex*, in press. **IF: 6.5**

2. **Heinzel, S.**, Metzger, F.G., Ehlis, A.C., Korell, R., Alboji, A., Haeussinger, F.B., Hagen, K., Maetzler, W., Eschweiler, G.W., Berg, D., Fallgatter, A.J., 2012. Aging-related cortical reorganization of verbal fluency processing: a functional near-infrared spectroscopy study. *Neurobiology of Aging*, in press. **IF: 6.2**

3. Hahn, T.*, **Heinzel, S.***, Plichta, M.M., Reif, A., Lesch, K.P., Fallgatter, A.J., 2011. Neurovascular coupling in the human visual cortex is modulated by cyclooxygenase-1 (COX-1) gene variant. *Cerebral Cortex* 21, 1659-1666. **IF: 6.5**

4. Haeussinger, F.B.*, **Heinzel, S.***, Hahn, T., Schecklmann, M., Ehlis, A.C., Fallgatter, A.J., 2011. Simulation of near-infrared light absorption considering individual head and prefrontal cortex anatomy: implications for optical neuroimaging. *PLoS One* 6, e26377. **IF: 4.4**

5. Hahn, T.*, **Heinzel, S.***, Dresler, T.*, Plichta, M.M., Renner, T.J., Markulin, F., Jakob, P.M., Lesch, K.P., Fallgatter, A.J., 2010. Association between reward-related activation in the ventral striatum and trait reward sensitivity is moderated by dopamine transporter genotype. *Human Brain Mapping* 32, 1557-1565. **IF: 5.9**

Publications as Co-author

6. Lesch, K.P., Selch, S., Renner, T.J., Jacob, C., Nguyen, T.T., Hahn, T., Romanos, M., Walitza, S., Shoichet, S., Dempfle, A., Heine, M., Boreatti-Hummer, A., Romanos, J., Gross-Lesch, S., Zerlaut, H., Wultsch, T., **Heinzel, S.**, Fassnacht, M., Fallgatter, A., Allolio, B., Schafer, H., Warnke, A., Reif, A., Ropers, H.H., Ullmann, R., 2011. Genome-wide copy number variation analysis in attention-deficit/hyperactivity disorder: association with neuropeptide Y gene dosage in an extended pedigree. *Molecular Psychiatry* 16, 491-503. **IF: 13.7**

7. Dresler, T., Ehlis, A.C., **Heinzel, S.**, Renner, T.J., Reif, A., Baehne, C.G., Heine, M., Boreatti-Hummer, A., Jacob, C.P., Lesch, K.P., Fallgatter, A.J., 2010. Dopamine transporter (SLC6A3) genotype impacts neurophysiological correlates of cognitive response control in an adult sample of patients with ADHD. *Neuropsychopharmacology* 35, 2193-2202. **IF: 8.0**

8. Hahn, T., Dresler, T., Ehlis, A.C., Plichta, M.M., **Heinzel, S.**, Polak, T., Lesch, K.P., Breuer, F., Jakob, P.M., Fallgatter, A.J., 2009. Neural response to reward anticipation is modulated by Gray's impulsivity. Neuroimage 46, 1148-1153. **IF: 5.9**

9. Plichta, M.M., **Heinzel, S.**, Ehlis, A.C., Pauli, P., Fallgatter, A.J., 2007. Model-based analysis of rapid event-related functional near-infrared spectroscopy (NIRS) data: a parametric validation study. *Neuroimage* 35, 625-634. **IF: 5.9**

Submitted

1. **Heinzel, S.***, Haeussinger, F.B.*, Hahn, T., Ehlis, A.C., Fallgatter, A.J.: Variability of (functional) hemodynamics as measured with simultaneous fNIRS and fMRI; *submitted.*

2. Hahn, T., **Heinzel, S.**, Notebaert, K., Dresler, T., Reif, A., Lesch, K.P., Jakob, P.M., Windmann, S., Fallgatter, A.J.: The tricks of the trait: Neural implementation of personality varies with genotype-dependent efficiency of serotonergic neurotransmission; *submitted*.

3. Plichta, M.M.*, **Heinzel, S.***, Gerdes, A.B.M., Ehlis, A.C., Schecklmann, M., Reif, A., Erdfelder, E., Dan, I., Tsuzuki, D., Lesch, K.P., Grön, G., Pauli, P., Fallgatter, A.J.: Impulsivity-related prefrontal activation during Reward Discounting is moderated by *COMT* Val^{158}Met polymorphism; *submitted*.

List of major conference contributions

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- Invited talk at the "Human Brain Mapping" Conference 2010 (Barcelona, Spain)
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- Interacademic Symposium of the National Academy of sciences and the Israel Academy of Science and Humanitites 2011 (Würzburg, Germany); Poster
- Forum of European Neuroscience (FENS) 2010 (Amsterdam, Netherlands); Poster
- Dresden Spring School 2010: From vulnerability to resilience: Molecular genetic perspectives; Poster
- Neurobiology doctoral student workshop (NeuroDoWo) 2009 (Würzburg, Germany); Talk
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  Quantitative comparison of methods measuring hemodynamic responses – a simultaneous fMRI and fNIRS-study.

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06/2009 – Practical and analysis supervision of medical doctoral theses

- Heiner Gieseke (Cand. med.): fNIRS, ADHD, *COMT*-genotype, delay discounting

- Robert Korell (Cand. med.): TREND-study, fNIRS, demographics

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