Imaging functional brain connectivity : pharmacological modulation, aging and Alzheimer's disease

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Chapter 1

General introduction

INTRODUCTION

Brain function relies heavily on neural communication and connections. Better understanding of the mechanisms that are related to maintenance or deterioration of brain function requires a technique that takes into account the elaborate nature of the central nervous system (CNS). A useful method for that purpose is to assess the brain's functional connectivity. Brain regions are functionally connected to each other when they exhibit correlating activation patterns [1], illustrating the complex organization of neural networks. Interactions between regions and within networks largely depend on chemical transmission between neurons. Functional connectivity might therefore also be regarded as representative of healthy or affected neural transmission that accompanies aging or neurodegeneration as seen in Alzheimer's disease (AD). The work as described in this thesis is aimed at elucidating the neural connections and serotonergic and cholinergic neurotransmitter pathways in healthy young and older adults and patients with AD.

Functional brain networks

Functional magnetic resonance imaging (fMRI) of the brain measures the change in the blood-oxygenation-level dependent (BOLD) signal over time for each voxel [2]. Based on the premise that active neurons consume more oxygen, the BOLD signal is considered to correspond to functional activity in these areas. More specifically, increased blood flow towards active brain regions alters the ratio of oxygenated to deoxygenated haemoglobin, leading to changes in MR images that are sensitive to the level of deoxyhaemoglobin [3, 4]. The term functional connectivity refers to temporal correlations between remote neurophysiological events [1]. Resting state fMRI (RS-fMRI) has been discovered as a suitable, non-invasive approach to study whole brain functional connections during rest as opposed to during the performance of an active task. Investigating resting state networks instead of task-related regional changes in activation has several advantages. With a relatively short duration of scanning sessions and the absence of complicated task instructions, it is more easily employed within specific patient populations and without the danger of different performance strategies. In addition, task fMRI is restricted to limited flexibility of designs and does not take into account the large-scale and interacting nature of the CNS [5].

Covariation of spontaneous low-frequency fluctuations in the BOLD response seems to reflect functional network interactions [6-8]. Even cortical regions that are spatially distant from each other can belong to the same functional network when its intrinsic activity is temporally coherent. Several resting state networks have consistently been detected in the brain and related to specific functions as visual, auditory and salience processing, motor performance and executive control [9, 10]. A typical resting state network is the default mode network (DMN), comprising the precuneus, posterior cingulate, temporal, parietal and medial prefrontal cortex [11, 12]. The

DMN is one of the most robust resting state networks that shows increased activation during rest as opposed to during the performance of active tasks, and is known to be involved in self-reference, introspection and episodic memory [13, 14].

Functional connectivity in aging and Alzheimer's disease

Measurements of brain network connectivity are increasingly being implemented as a means to investigate neurologic and psychiatric disease [6]. AD, the most common cause of dementia among older adults, is a neurodegenerative disease with a slow onset that progressively affects multiple cognitive domains. It is primarily associated with a deterioration of memory, and additional loss of functions related to language, orientation in time and place, visuospatial and executive functioning as well as changes in personality and behavior [15]. One of the major risk factors for AD is advancing age and because of the aging population, the number of patients with AD is expected to keep on rising considerably [16, 17]. The increased prevalence of AD and the burden it poses on health care and the daily life of patients and their proxies calls for development of early diagnostic markers and improved insight into disruption of neural processes. Examination of altered connectivity is especially relevant since AD is nowadays recognized as a disorder of vanished functional connections [18, 19].

AD is mainly characterized by reduced hippocampal and DMN connectivity [20, 21]. A few studies show that frontoparietal, visual, executive, sensory, motor, cerebellum/basal ganglia and salience networks are affected in AD as well, with evidence for increases as well as decreases in connectivity [22-26]. Although exact causal mechanisms are not completely understood, connectivity alterations are possibly representative of decrements in metabolism [27], white matter integrity [28-30], amyloid deposition [31] and structural atrophy [18, 32]. In line with findings on connectivity disruptions, these hallmarks are frequently found at regions within the DMN and the interrelated hippocampus. Functional connectivity abnormalities have also been found in patients suffering from mild cognitive impairment [33, 34] and healthy subjects with a genetic risk for the development of AD [35, 36]. But 'normal' aging appears to have an influence on DMN coherence as well, as connectivity with anterior and posterior components is compromised in healthy older adults [20, 37]. An age-appropriate degradation in mental functioning might be associated with this decline in DMN connectivity [28, 37]. Studies on the effect of aging on other functional networks are scarce, but indicative of reduced connectivity with dorsal attention, salience, motor, somatosensory and visual networks [28, 38-42].

Neurotransmitter systems in aging and Alzheimer's disease

Apart from a decline in functional connectivity, aging and Alzheimer's disease are characterized by decreased neurotransmitter functioning [43-45]. Neurotransmission is crucial for communication

between neurons and consequently affects behavior and mental processes. Two neurotransmitters that are implicated in both aging and AD are serotonin (5-hydroxytryptamine; 5-HT) and acetylcholine [46]. The serotonergic system comprises a complex organization of pathways that influences the brain from early development through degeneration with aging. With 7 different main classes (5-HT_{1-7}) and 14 subclasses, not only present in the central nervous system but also in the digestive tract and blood platelets, serotonin influences a wide range of functions and is related to multiple mental, behavioral and physiological states [47, 48]. Serotonin is a monoamine that is released by the dorsal and ventral raphe nuclei in the midbrain towards a vast part of the cortex, limbic areas, hypothalamus, basal ganglia, brain stem and cerebellum [49, 50]. In aging and AD, diminished serotonin activity, as demonstrated by reduced pre- and postsynaptic binding, is mostly associated with changes in behavior and the increased prevalence of depression and mood disorders [45-47, 51]. Selective serotonin reuptake inhibitors (SSRIs) are commonly used as medication for depression and anxiety disorders. SSRIs prevent the natural reuptake of serotonin by presynaptic receptors, thereby increasing the available amount of serotonin to postsynaptic terminals that leads to prolonged action on adjacent neurons [52].

The neurotransmitter acetylcholine has been related to changes in cognitive performance in both normal aging and AD [53, 54]. Cholinergic receptors are subdivided into the muscarinic (M) and nicotinic (N) families, together consisting of 17 subclasses that are located in the central and peripheral nervous system, at the neuromuscular junction, the heart and smooth muscles [55-57]. In the CNS, acetylcholine is primarily synthesized in the basal forebrain and binds to receptors that are present throughout the cortex, thalamus, amygdala and hippocampus [58, 59]. A reduction of choline acetyltransferase and acetylcholinesterase activity in AD [60-62] is explained by the notion that basal forebrain atrophy and associated neuronal loss leads to reduced transmitter release towards the cortex, hippocampus and thalamus, hence affecting memory and attentional processes [63, 64]. It is possible that the cholinergic system integrity is compromised by the deposition of amyloid-β and formation of tangles in AD, which show a high density at locations of neuronal and synaptic loss [65-67]. Acetylcholinesterase inhibitors (AChEIs) inhibit the hydrolysis of acetylcholine by the enzyme acetylcholinesterase, thereby increasing acetylcholine levels in the synaptic cleft. As treatment of cognitive symptoms, AChEIs are mainly useful in the early stages of AD when the decrease of neurotransmitter release is still limited [68, 69]. Although the effectiveness of reducing the breakdown of acetylcholine (or butyrylcholine) in AD is far from satisfactory, there are currently not many alternatives [70-72]. Despite the hypothesized cholinergic dysfunction in normal aging, there is minor evidence of cognitive improvement by AChEI treatment in elderly without AD [73].

Pharmacological challenge effects on functional brain connectivity

The concept of pharmacological challenges implies a promising approach to discover the underlying neurobiological mechanisms behind drug action and neurotransmitter-related disease [74]. This method uses the principles of a pharmacokinetic study with dosing of a drug, followed by a series of measurements at intervals predicted to be relevant for the particular drug being studied. Challenging the CNS with drugs that selectively alter central neurotransmission and are aimed at restoring synaptic connections is an efficient procedure to examine the role of neurotransmitters in healthy and pathological brain functioning [75]. Resting state networks are representative of interactions between different brain areas and therefore sensitive to changes in neurotransmission. Using RS-fMRI to measure challenge effects offers the possibility to visualize and localize patterns and deficits in neurotransmitter systems by imaging pharmacologically induced alterations of related pathways in the brain. An advantage of RS-fMRI measurements in pharmacological research is that they can be repeated frequently, which allows studies of network variations over limited time periods, with changing drug concentrations or diurnal fluctuations. RS-fMRI studies of challenges with dopamine (ant)agonists, morphine, ketamine, Δ9-tetrahydrocannabinol (THC) and ethanol have shown convincing agent-specific network responses [76-81]. In this thesis, we used the pharmacological challenge technique to investigate the serotonergic and cholinergic neurotransmitter systems by measuring acute SSRI and AChEI effects on resting state functional connectivity.

Single-dose and short-term administration of SSRIs has been demonstrated to mainly lower connectivity in healthy and depressed young subjects [82-87], which is in line with opposite observations of increased connections in depression [88]. Most of these studies restricted their analysis to the DMN, although Schaefer et al. [83] also show connectivity change in many cortical and subcortical regions outside this network. Long-term treatment with AChEIs in AD patients seems to enhance connectivity of DMN and hippocampal areas [89-94]. Single-dose effects on resting state connectivity and the action of AChEIs on functional connections in healthy brains are thus far not investigated but might provide additional knowledge on the role of acetylcholine in neural communication and functions. Likewise, the effects of SSRIs on resting state connectivity in elderly and AD patients are unknown but could improve our understanding of age- and AD-related changes in serotonin pathways. Since serotonergic and cholinergic neurotransmitter systems are known to be altered in AD, and RS-fMRI is known to be sensitive to pharmacological challenges, provoking these neurotransmitter systems with drug challenges combined with RS-fMRI was hypothesized to reveal greater differences between AD and non-diseased elderly compared to changes in functional connectivity alone. In that case, the challenge approach might even lead to a better differentiation of AD from healthy aging and, ideally, to an earlier diagnostic tool.

AIMS AND OUTLINE OF THIS THESIS

The main objective of this thesis was to investigate the serotonergic and cholinergic systems of the brain and the association of older age and AD with changes in neurotransmission and functional connections. The included studies were intended to provide more insight into neural trajectories in health and the way these are affected in aging and dementia. For that purpose, we examined patterns of functional connectivity using RS-fMRI with and without pharmacological challenges in healthy young and older adults, and patients with AD. In all studies we implemented a standardized and comprehensive method and design, by examining whole brain connectivity and functional change induced by serotonergic and cholinergic drugs and/or associated with age or disease. To assess these effects, we used RS-fMRI and the computerized NeuroCart® test battery, developed for quantifying CNS function with tasks and visual analogue scales measuring mood, alertness, calmness, memory, emotional processing, executive functioning and reaction time [95-103]. In case of pharmacological experiments, additional measures of pharmacokinetics, cortisol and prolactin were taken to monitor the drugs' absorption rate and investigate neurotransmitter function based on the neuroendocrine response, which may also be modulated by SSRIs and AChEIs [104-106]. Repeated pharmacodynamic measures (RS-fMRI, NeuroCart® and neuroendocrine parameters) after drug or placebo administration followed a strict time schedule conform the pharmacokinetic characteristics to ensure appropriate and standard measurements, and enable adequate comparison of results across and within studies. Several methods exist to measure functional connectivity, such as graph theory [107] and seed-based analyses [108]. In our studies, we applied a standardized approach to study functional network change by using ten predefined resting state components as networks of interest [10].

Part I: Pharmacological challenge effects on brain connectivity in healthy young adults

The first part of this thesis concerns two pharmacological studies that were executed in healthy young subjects. These studies were intended to determine the sensitivity of brain connectivity to serotonergic and cholinergic challenges, and to gain more insight into the mechanisms of drug action of SSRIs and AChEIs. **Chapter 2** describes the randomized, double blind, placebo-controlled, crossover study on the SSRI sertraline in 12 young volunteers. After drug or placebo intake, RS-fMRI scans and measures of cognitive and subjective functioning were repeatedly collected. Multiple blood samples were taken as well to define neuroendocrine and pharmacokinetic levels. In **chapter 3**, we further investigate acute pharmacological effects in young volunteers. The SSRI citalopram and the AChEI galantamine were administered to 12 subjects that were matched for age and gender with the subject group of chapter 2. A randomized, double blind, placebo-controlled, crossover design was used with repeated measures before and after drug or placebo intake, including blood samples, NeuroCart® task performance and RS-fMRI.

Part II: Functional brain connectivity and neuromodulation in older age and Alzheimer's disease

The second part of this thesis was aimed at discovering changes in brain connectivity and serotonergic and cholinergic systems in old age and AD. The results of these studies may lead to a better understanding of neurotransmitter system decline, and the possible rehabilitative effects of SSRIs and AChEIs in old age and AD. **Chapter 4** includes the outcomes of a study that was conducted to investigate differences in resting state functional connectivity without pharmacological modulation between 12 young and 12 elderly adults and 12 patients with AD. To examine whether functional connectivity changes might be (partially) explained by atrophy of brain structure, the results of this study were presented with and without correction for local gray matter volume. In the final two chapters, effects of an SSRI and AChEI on brain connectivity were compared between young and older adults and between older adults and AD patients. In **chapter 5** we present the results of a randomized, placebo-controlled, double blind, crossover study in 12 young and 17 older adults. The effects of the SSRI citalopram and the AChEI galantamine on resting state connectivity and task performance were compared between young and older adults. Outcome measures (RS-fMRI, blood samples and NeuroCart® task performance) were repeatedly taken before and after drug or placebo intake. The same design was used for the study as described in **chapter 6**, which shows the results of a comparison between 12 patients with AD and 12 elderly controls after a serotonergic and cholinergic challenge.

To conclude, a summary and general discussion of the presented results and future perspectives are provided in **chapter 7**.