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

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Chapter 7

Summary and general discussion

SUMMARY

The purpose of this thesis was to investigate the brain's serotonergic and cholinergic systems, and the way these are altered in older age and AD. The effects of pharmacological challenges on functional brain networks as measured with resting state fMRI (RS-fMRI) may provide us more insight into neurotransmitter pathways and mechanisms of action of drugs that selectively act on the central nervous system (CNS). In addition, it was examined how functional brain connections change in aging and AD. By comparing network connectivity and the pharmacological response of this measure between healthy young and older subjects and patients with AD, we aimed to improve knowledge on the decay of brain function and neurotransmission associated with normal aging and AD. Better understanding of these (patho)physiological systems is of substantial importance considering the ongoing increase in life expectancy and AD incidence [16, 17].

In the first part of this thesis we explored the responsiveness of brain connectivity to a single-dose of the selective serotonin reuptake inhibitors (SSRIs) sertraline and citalopram and the cholinesterase inhibitor (AChEI) galantamine, and its sensitivity compared to other outcome measures in healthy young volunteers. In the second part we studied functional connections and neurotransmitter systems in old age and AD. It was examined how age-related connectivity changes compare to changes as found in AD, and whether these are dependent of structural atrophy. We thereafter investigated if network alterations after modulation with citalopram and galantamine differ between young and elderly subjects, and between elderly controls and patients with AD. The present chapter provides a summary and discussion of the main findings of the included studies and recommendations for future research.

In **chapter 2** the effects of a serotonergic challenge compared to placebo on whole-brain connectivity were measured in healthy young subjects. The single-dose administration of the SSRI sertraline caused large-scale connectivity alterations with multiple networks: the default mode network (DMN), the executive control network, the lateral, occipital and medial visual networks, the sensorimotor network and the auditory network. Regions that were most consistently affected among these network changes were the precuneus and anterior and posterior cingulate cortex (ACC and PCC). Sertraline did not alter cognitive or subjective measures that were taken by means of computerized cognitive tests and visual analogue scales to examine changes in mood, alertness, calmness, memory, emotional processing, executive functioning and reaction time. The results of this study demonstrate the widespread nature of the serotonergic system, its involvement in regions and networks that relate to emotional and sensory processing and motor control, and the sensitivity of RS-fMRI to a serotonergic challenge.

In **chapter 3** we assessed the acute effects of the SSRI citalopram and the AChEI galantamine vs. placebo on brain connectivity in young subjects. Citalopram mainly resulted in decreased connectivity within and between the sensorimotor network and areas that belong to the DMN.

Galantamine enhanced network connectivity of the medial visual network with regions as the fusiform gyrus, hippocampus, PCC and thalamus. There were no significant treatment effects on cognitive test performance or subjective states. Citalopram effects showed a clear overlap with those of sertraline and indicate that serotonin might be related to motor behavior and self-referential mechanisms. Connectivity alterations due to galantamine point to a role of acetylcholine in visual processing, learning and memory. These findings further support the use of RS-fMRI as a specific and sensitive method to measure pharmacological challenge effects.

Differences in functional network connectivity between young and elderly subjects and patients with AD were investigated in **chapter 4**. It was concluded that differences between young and older adults are considerably larger than between AD patients and elderly controls. Both comparisons point to reduced brain connectivity in old age and AD. Older age was associated with widespread diminished connectivity with nine of the ten investigated functional networks. In AD patients compared to controls, the DMN was the only network that showed reduced connectivity. When the results were corrected for regional gray matter volume, the effects were maintained but markedly attenuated (showing 58-65% loss in number of voxels with a significant effect). The findings suggest that decreased DMN-precuneus/PCC connectivity, known to be related to visuospatial functioning, episodic memory and self-consciousness, may act as a marker of AD.

In **chapter 5**, the effects of serotonergic and cholinergic challenges on network connectivity were compared between young and older adults. We showed that the SSRI citalopram reduced sensorimotor network connectivity in both young and elderly subjects. Although this decrease in connectivity was more abundant in the young subject group, citalopram did not lead to a significant group x treatment interaction effect. The drug-induced network response to galantamine was significantly diminished in elderly compared to young subjects. Whereas the young subjects showed increased connectivity as described in chapter 3, we did not find any network alterations in the elderly. There were no notable differences between groups in effect on subjective and cognitive functioning. The results indicate that older age is accompanied by a relatively unaltered serotonergic system and a decline in cholinergic neurotransmission.

Differential effects of serotonergic and cholinergic modulation in AD patients and controls were examined in **chapter 6**. There were no significant differences between groups in citalopram effects on network connectivity, although the observed effect on the sensorimotor networks as found in the elderly subjects did not reach significance in the AD group. Further, a citalopram-induced increase in DMN-precuneus/PCC connectivity was observed in AD patients, but not within the group of elderly controls. A significant difference after cholinergic enhancement was found between groups in connectivity with the cerebellar network. Galantamine did not alter functional connectivity in the elderly subjects, but reduced connectivity within the cerebellar network and in relation to the thalamus and brain stem in AD patients. The results did not reveal any convincing group x treatment interaction effects on cognitive and subjective measures.

These findings suggest that serotonergic enhancement might reverse reduced DMN-precuneus/PCC connectivity as seen in AD. The effects of galantamine point to involvement of cerebellar connections in cholinergic system alterations in AD.

GENERAL DISCUSSION

We showed that neuromodulatory effects on brain connectivity were most noticeable in healthy young subjects. Further, although the effect of older age on network connectivity (without pharmacological stimulation) was substantial, the differences in connectivity between AD patients and controls were limited. Whereas the network response to serotonergic stimulation was not significantly different between young and older adults and patients with AD, cholinergic enhancement induced a differential response in both elderly and AD patients, which might relate to the cholinergic hypothesis stating that the decay of the cholinergic system plays a role in the cognitive decline in aging and AD [53]. The findings of this thesis indicate that RS-fMRI offers a sensitive method to investigate acute pharmacological effects on the serotonergic and cholinergic systems, compared to several cognitive and subjective measures. Many results of the implemented pharmacological challenge studies were convincing with regard to location and direction of effect. This substantiates the usefulness of RS-fMRI as a method for measuring a compound's mechanism of action and its possible value in CNS drug development.

SSRI effects on brain connectivity in healthy young subjects

The results in chapter 2 and 3 show that the SSRIs sertraline and citalopram changes network connectivity in healthy young subjects. To allow for comparison of the effects of both SSRIs in healthy young subjects, the inclusion criteria and design of the sertraline study (chapter 2) were set up in agreement with the citalopram study. The twelve subjects of chapter 2 were each individually matched for age and gender with the young subjects as included in chapter 3. Despite the use of equipotent doses, the effects of sertraline on connectivity were more excessive compared to citalopram. However, the direction and areas of connectivity change were quite similar which aids the reliability of the RS-fMRI technique to show comprehensible pharmacological effects. Both SSRIs induced a decrease in connectivity with the sensorimotor network, midbrain and cortical midline structures as the precuneus, ACC, PCC and medial prefrontal areas. These effects suggest that serotonin is involved in motor function [221, 222], self-consciousness and emotion regulation [219], and the integration of sensory, motor, cognitive and emotional information [141, 368]. It has been proposed that increased brain connectivity of midline regions as seen in depression [88] represents increased self-consciousness and rumination with negative thoughts [142, 144]. Our findings therefore imply that SSRIs might reverse depression-related connectivity patterns.

Slight adjustments were made to the time schedule as sertraline and citalopram differ in their pharmacokinetic profile. In addition, we replaced the Visual Verbal Learning Test (VVLT) by the Face Encoding and Recognition task. The absence of symptom relief after short periods of SSRI treatment in patients suffering from depression or anxiety disorders might be explained by the fact that improved emotional processing can only be made visible by tests that evoke an affective state [173, 174]. Accordingly, the Face Encoding and Recognition task was hypothesized to induce a larger acute response than the VVLT. However, sertraline and citalopram did not affect performance on any cognitive or subjective test compared to placebo. A significant increase in neuroendocrine levels after SSRI administration implies that these unaltered measures could not be directly attributed to insufficient dosages or a lack of statistical power. It is possible that this paucity of effect is the consequence of inhibition of 5-HT_{1A} autoreceptors in the raphe nuclei during the acute stages of SSRI treatment [134]. The results of both SSRI studies verify that serotonergic tracts cover a substantial part of the brain and demonstrate the sensitivity of RS-fMRI to pharmacological challenges compared to other pharmacodynamic outcome measures.

Functional networks and neuromodulation in old age

By comparing three groups (young and older adults and patients with AD), we aimed to obtain better founded information about the effect of age vs. AD on functional network coherence and neurotransmitter systems. First, differences in network connectivity between young and older adults and between elderly and AD patients were compared in one study (chapter 4). The results of this study indicate that older age affects connectivity more extensively than AD. Of all ten investigated networks, nine showed diminished connectivity in the older adults compared to young subjects. These widespread reductions were not fully explained by loss of structural brain volume, as part of the findings was maintained after accounting for local differences in gray matter. Reduced connectivity was found for the DMN and for networks relating to language, attention, visual, auditory, motor and executive functioning, which may represent the age-appropriate decline in cognitive, sensory and motor function [254-256].

Despite the widespread loss of functional network connections at older age, the serotonergic system seemed relatively unaltered as the pharmacological effects of citalopram on brain connectivity did not differ between young and older adults (chapter 5). In both groups, citalopram caused a significant reduction of sensorimotor network connectivity. Although the citalopram induced response appeared smaller in older adults, a group x treatment interaction did not reach statistical significance. Serotonin dysregulation at older age could be related to the increased prevalence of depression in the elderly [45, 47, 310] and the similarity in response between young and old subjects is possibly the consequence of the absence of mood disorders in the older adults that were included in our study. The network response to galantamine in elderly subjects was indicative of cholinergic change at older age. In the young subjects, increased

occipital visual network connectivity was observed after galantamine with the hippocampus, precuneus, thalamus, fusiform gyrus, precentral and superior frontal gyrus, PCC and cerebellum. These specific alterations point to the role of acetylcholine in functions as learning, memory, and visual perception and processing [229, 233, 234]. In contrast, no change was found in the elderly, which was hypothesized to be the consequence of a cholinergic system decline that is characteristic for the process of normal aging and has been related to cognitive decay [329].

Functional networks and neuromodulation in Alzheimer's disease

In AD, altered network coherence was restricted to decreased DMN-precuneus connectivity (chapter 4). This is not an unexpected result and suggests a consistent and specific hallmark of AD [20, 23, 284], possibly relating to memory problems and visual-spatial symptoms [159, 160]. Our observations also indicate that functional network coherence is more affected in older age than in AD. After correction for regional GM volume the difference between AD patients and elderly controls was less profound but still involved a decrease in connectivity of the DMN with the precuneus. Measuring the effects of serotonergic and cholinergic challenges with RS-fMRI could offer us new insights into neurotransmitter system functioning and the potentially rehabilitative mechanisms of SSRIs and AChEIs in AD (chapter 6). Although we did not find any statistically significant differences between AD patients and controls in the network response after citalopram administration, the observed reduction in sensorimotor network connectivity in the elderly was not found in the group of AD patients. This might indicate the presence of a decline in serotonin networks that could not be determined with the small-sized cross-sectional studies that we performed. Figure 7.1 shows the citalopram effects on sensorimotor network connectivity in the three investigated groups.

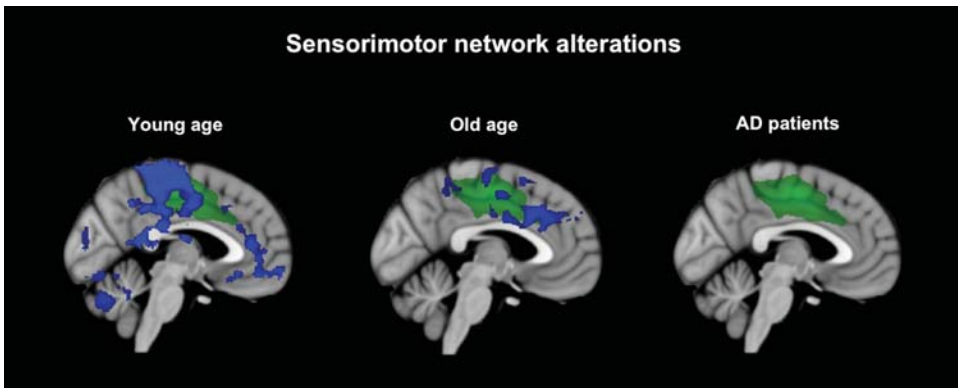

Figure 7.1. Reduced connectivity after citalopram administration between the sensorimotor network (green) and areas as shown in blue in young and older adults and patients with AD (chapter 3, 5 and 6, respectively).

The observed diminished DMN-precuneus connectivity in AD likely relates to deterioration of episodic memory, self-consciousness and visuospatial performance [159, 160]. Therefore, an interesting finding was the increase in these connections after citalopram intake. This observation seems to be fairly specific for AD as it differs from our results in healthy young and older subjects that consistently showed reduced network connections after single-dose SSRI administration. Decreased connectivity after SSRI administration has also been found in other studies that investigated SSRI effects in healthy young subjects [82, 83, 85-87] and patients with a major depressive disorder [84]. Apparently, SSRIs have a differential effect in AD patients compared to healthy or depressed subjects. The resemblance in location of the opposite findings as found in chapters 4 and 6 (see Figure 7.2) is of interest as it could signify a partial restoration of some aspects of AD associated pathology [159] by SSRIs.

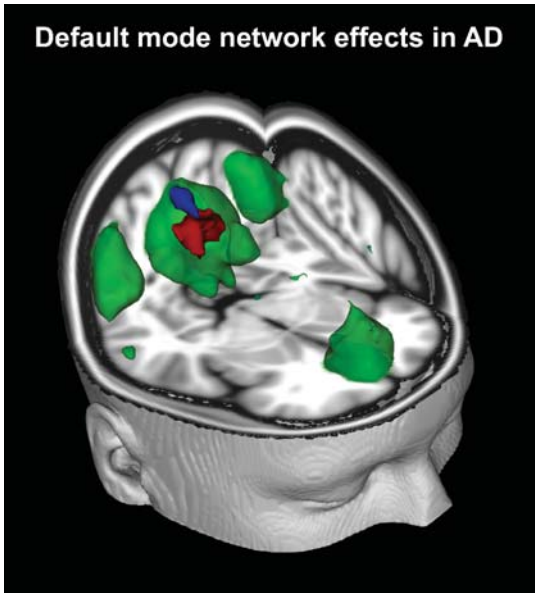

Figure 7.2. The DMN (green) shows a decrease in connectivity with the precuneus (red) in AD (chapter 4) as opposed to an increase in connectivity (blue) after citalopram administration in AD (chapter 6).

The increased functional connections with the visual network after galantamine as found in the young subjects were absent in AD patients as well. In addition, we found alterations that were specific for AD (Figure 7.3). An unexpected finding in chapter 5 was a galantamine induced increase in connectivity within the cerebellar network and between this network and the thalamus and brain stem. Although the thalamus is known to contain many acetylcholine receptors that receive input from a prominent cholinergic cell group in the brain stem [54, 358],

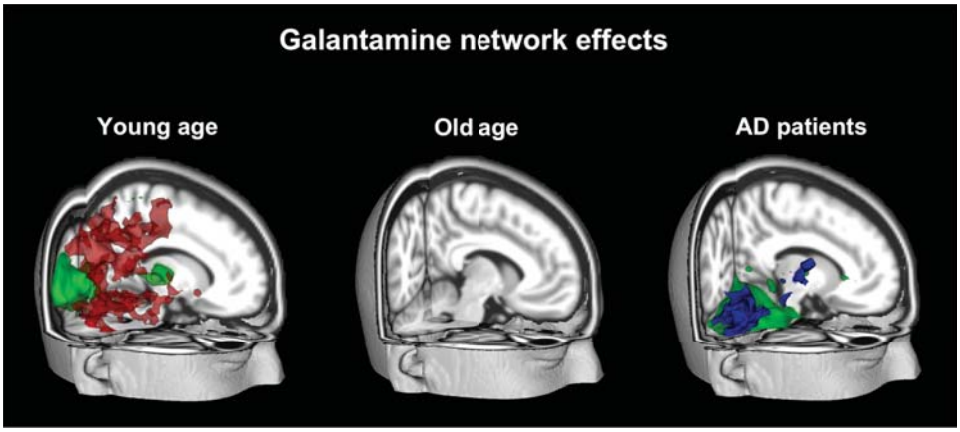

Figure 7.3. Galantamine effects on network connectivity. In the young adults, galantamine increased connectivity between the occipital visual network (green) and regions as shown in red (chapter 3). Galantamine did not alter connectivity in the older adults (chapter 5). AD patients showed a decrease in connectivity between the cerebellar network (green) and regions as shown in blue (chapter 6).

it is not straightforward to relate cerebellar connectivity change to cholinergic challenge effects. However, the cerebellum is known for a high activity of acetylcholinesterase, and acetylcholine seems to excite the cerebellum's muscarinic Purkinje cells and mossy fibres that are rich in choline acetyltransferase [349-353] and have been shown to be depleted in AD [356].

Differentiation of older subjects from patients with AD became more clear after challenging the cholinergic system, which made between-group differences in brain connectivity visible that are otherwise not apparent during rest. However, although the area was larger, this response was still restricted to one network and regions that are not typically involved in cholinergic transmission and AD. The results of this study do not directly have clinical implications for the field of dementia but they do seem to provide novel and fundamental knowledge about aspects of system decline related to AD. In our study a relatively low dosage of galantamine (8 mg) was used and it is likely that a 16 or 24 mg dosage could reveal larger and more robust differences between groups. More research is needed to replicate our findings and investigate the possible role of cerebellar network connections in relation to cholinergic decline in AD.

Strengths and limitations

A major strength of the pharmacological studies that we performed is the applied randomized, double blind, placebo-controlled, crossover design with multiple outcome measures that were acquired before and after drug administration. By collecting not only RS-fMRI data, but also cognitive and subjective measures and blood samples, we obtained a unique dataset that

allowed us to examine and discuss the results in more detail. Pharmacodynamic measurements were repeatedly obtained post dosing at time points when, based on the known T_{max}, the largest effects were expected. This design resulted in several advantages, as it made it possible to 1) inspect the pharmacokinetic profiles and neuroendocrine levels that could reassure us of sufficient absorption and choosing appropriate time points of measurements, 2) collect large datasets to increase the power of statistical tests despite small sample sizes, 3) explore diurnal fluctuations in resting state connectivity on placebo days and compare these to changes after drug administration, 4) determine the sensitivity of RS-fMRI to pharmacological challenges compared to other outcome measures, and 5) investigate differences in connectivity between groups before (chapter 4) and after pharmacological stimulation (chapter 5 and 6).

Nevertheless, our results cannot be generalized, as the small subgroups are likely not representative of larger populations. The included participants were all motivated to participate and selected based on strict inclusion and exclusion criteria. Another consideration is the chance of including 'healthy' elderly in a preclinical stage of Alzheimer's disease or another type of dementia, which was at the time of recruitment not yet identified. Likewise, although all patients have been carefully screened and diagnosed with probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [15], we cannot exclude the possibility that some of the included AD patients have been misdiagnosed. All patients were diagnosed very recently and especially in the early stage of the disease, clinical symptoms and biomarker alterations of different dementia types often overlap [369]. Further, although analysis of functional connectivity appears to be an informative method to investigate neurotransmitter system function, RS-fMRI cannot be used to measure neurotransmission directly. Functional connectivity is a mathematical concept of correlations between BOLD signals of different brain areas, which are not easy to interpret, prone to physiological noise such as breathing and heart rate [126], and not representative of causal or directional change [370].

Future research

In this thesis, we investigated acute drug effects on functional connectivity using RS-fMRI. Simultaneous fMRI and positron emission tomography (PET) or arterial spin labeling (ASL) acquisition could aid the interpretation of resting state fMRI results by identification of chemical pathways and the vascular pharmacological response. The advantage of PET over fMRI is that biochemical processes can be measured directly at the receptor level by localizing specific ligands in vivo. Ligand radiolabeling offers the possibility to measure receptor occupancy and trace the dispersion and binding of a drug in the brain [371]. But the duration, expensiveness and radioactive exposure form large drawbacks of PET imaging, making it practically impossible to execute studies with multiple repeated measures as implemented in our design. ASL, a method

to measure cerebral perfusion, is not hindered by these problems. Investigation of variations in blood flow after pharmacological modulation might be of additional value in CNS drug research.

An advantage of our repeated measures design was the possibility of examining drug effects on different time points post dosing for a detailed investigation over time in relation to pharmacokinetic properties. A challenge for the future involves PK/PD-modeling, the mathematical procedure to relate individual pharmacokinetic profiles of a drug to pharmacodynamic outcome measures [372]. Although we explored this relationship by determining the absorption rate of sertraline, citalopram and galantamine and inspecting time related effects in more detail in chapter 3, PK/PD-models are not yet available for voxelwise fMRI data. A formal model to quantify the dose-response relationship will give more insight into the value of RS-fMRI as a measure of pharmacological effects that might vary with different drug concentration levels.

We cannot draw firm conclusions about the clinical usefulness of pharmacological RS-fMRI as a means to distinguish between AD patients and controls. More research needs to be conducted to explore this possibility in detail and investigate whether drug challenge responses may also discriminate between other forms of dementia, such as frontotemporal dementia, dementia with Lewy bodies and vascular dementia. The use of pharmacological data in individual statistical classification and machine learning, and comparison with markers that are known to be most sensitive to AD might shed more light on the value of implementing pharmacological challenge paradigms as part of diagnostic procedures. For example, the inclusion of our data may improve diagnostic classification of AD [373]. Another interesting approach of the pharmacological challenge technique as applied in this research would be to examine whether the initial network response to an SSRI or AChEI could be predictive of long-term treatment effectiveness [374-376].

Conclusions

The present research suggests that older age is characterized by widespread decreases in functional network connections, which is not totally dependent of structural atrophy. This might represent the fact that normal aging is accompanied by a decline of several functions (vision, hearing, motor behavior, language, executive and cognitive function) [254-256]. The serotonergic system does not necessarily deteriorate at older age, whereas the cholinergic system shows diminished responsiveness, relating to regions and networks that are involved in memory and visual processing. Furthermore, we replicated the finding of reduced DMN connectivity in AD, and showed that this was only partly explained by loss of structural volume. Our data also indicate that the decrease in connectivity in old age is considerably larger than connectivity decline in AD. Although there was no proof of serotonergic network change in AD vs. elderly controls, our results point to an attenuation of serotonin pathways in old age and AD. In addition, the reduced DMN-precuneus connections in the AD patients are partly reversed after a citalopram challenge.

The effects of a galantamine challenge on the cerebellar network imply that cholinergic pathway disruptions in AD are related to cerebellar-thalamic connections.

Consistent with our findings, SSRIs or AChEIs do not typically alleviate the cognitive or behavioral symptoms of mood disorders and dementia immediately after drug administration [173, 174]. Pharmacological companies are therefore in search for more sensitive measures that might show specific changes even after single-dose administration. Functional connectivity analysis appears to be a promising technique in investigating brain function and neurotransmitter pathways after a pharmacological challenge. The largest pharmacological effects were found in healthy young volunteers, which might be the consequence of relatively uncompromised neurotransmitter systems. More research is needed to determine the potency of RS-fMRI to examine the way that neurotransmitter pathways are altered in normal aging, AD or other neuropsychiatric disorders. The results of our studies suggest that functional brain connectivity might serve as a sensitive and specific measure in pharmacological research, and possibly as a tool to predict treatment success in patient populations and/or characterize novel compounds under development. Future pharmacological imaging research may be improved by the integration of different imaging modalities such as PET and ASL, and the addition of PK/PD-modeling.