Corpus callosum in aging and dementia

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LIST OF ORIGINAL PAPERS


INTRODUCTION

SETTING THE STAGE FOR THE THESIS – A HISTORICAL PERSPECTIVE

At least as early as the 1500s, anatomists clearly distinguished between white and grey matter of the cerebrum, and with the invention of the microscope in the 1600s, the fibers of the white matter were observed [1]. Despite these and other observations of the salient features of white matter structure, the first clinicopathological investigations of neurological deficits focused on the cortex. Broca’s seminal observation of the importance of the left frontal cortex for language production [2], helped promote the corticocentric idea of cortical localizationism, i.e. that cognitive functions reside in specific cortical areas [1,3]. To underline what retrospectively can only be viewed as disproportionate emphasis on the cortex and relegation of the white matter, it was proposed that the purpose of the largest fiber bundle of the brain, the corpus callosum (CC), was mechanical in that it prevented the brain from sagging [4]. However, not all researchers considered the white matter to be without importance in respect to cognitive function. Wernicke, in the late 1800s, predicted the existence of a speech impairment due to a lesion in the white matter which would disconnect the motor language area (Broca’s area) from the sensory language area (the superior temporal gyrus, later Wernicke’s areas), termed conduction aphasia [5]. This laid the foundations for the associationist theory of the brain, which was based on the assumption that all cognitive functions of the brain was a result of the connectivity of the brain [6]. Following, a series of neurological syndromes (e.g. [7,8]), which were to be termed classical disconnection syndromes, were described. In 1892, Dejerine described what is still considered to be the epitome of callosal syndromes, namely pure alexia without agraphia, due to a lesion in the CC [9]. However, the ideas of associationist theory had more or less disappeared out of the science of neurology by the early 1900s, and was eclipsed by cortical localizationism [6,10] and the theory of equipotency [11]. A renewed interest in white matter connectivity and its role in cerebral functions was motivated by a landmark publication in 1965 by Geschwind describing various disconnection syndromes in animals and humans [12,13]. By doing so, Geschwind introduced a theoretical framework where cognitive functions were reliant on specific networks in the brain. Geschwind built his paper on the work by Wernicke and others, but also extended it. For example, according to Geschwind, lesions in cortical areas were also hypothesised to be able to cause disconnection syndromes, a proposition based on the 19th century observation, that no primary sensory cortical areas directly connected with other distant cortical areas [14]. Instead connections only range to adjacent association cortex, which acts as a relay station. Moreover, the theoretical considerations of Geschwind when compared to Wernicke’s, were more compatible with the lesions and resultant deficits seen in the clinic, and importantly enabled the formulations of hypotheses which were readily testable [15]. What may be said to have emerged following Geschwind’s landmark paper has not been to position white matter as the dominant structural underpinning of cognitive functions. Rather, it paved the way for a model which juxtaposes grey and white matter, and emphasises activity in distributed neural networks as the basis of the cognitive functions of the brain. A “connectionist”
model in which the cognitive behavioral output represents an emergent property of the network, not just the additive product of the cortical areas connected within the network [16,17]. One interpretation of the implicit consequences of this model is that the functions of the grey matter at a given time is dictated by the network by which they are currently engaged. Therefore, if the functional connectivity in the network has a structural underpinning, the white matter may be said to dictate the functional state of the grey matter structures. Along with this model has also come the realisation that the classical disconnection syndromes, which related a single relatively well-defined lesion to the appearance of a syndrome, does not suffice to explain how more prevalent pathologies affecting the white matter may lead to less well defined syndromes and deficits [18]. To take an example relevant to this thesis, dementia may indeed ensue from a single lesion in the white matter such as a strategic infarct. However, diffuse and wide-spread pathology leading to disruption of the white matter may be a more prevalent type of white matter lesion leading to dementia [18]. The research into the structural correlates of dementia have had a similar “corticocentric” bias as previously outlined for cognitive functions in general. It is unequivocal that Alzheimer’s disease (AD), the most prevalent neurodegenerative cause of dementia, is associated with cortical atrophy, including hippocampal atrophy. However, imaging studies have shown that changes in the white matter also occurs [19]. Therefore, the premise of this thesis is that neurologic functions including cognitive functions are reliant on intact white matter, and that structural changes in the white matter, including the CC, may play a role in dementia and age-related functional and cognitive decline.

THE WHITE MATTER

The two major tissue types of the brain are grey and white matter respectively with white matter occupying approximately 40-50 % of the total brain volume [20]. The cortical mantle and subcortical nuclei consists of grey matter, whereas white matter is interspersed between these structures. On MRI and neuropathological examination, white and grey matter may appear as two distinct but uniform tissue types, but does not surprisingly consist of different cellular components. In the white matter, discrete ensembles of axons, termed fiber tracts or bundles are found. The axons arise from neuronal cell bodies located in the grey matter from where they project into the white matter. Axons in the white matter consists of mostly myelinated axons, i.e. axons ensheathed by many layers of oligodendrococyte membrane. The fatty myelin sheath is the source of the white appearance of this tissue type, and its principle function is to modify the electrical property of the axon, conferring a much higher propagation of the action potential and reducing the refractory period following an action potential before a second may be conducted [21]. Myelinization of the brain is far from completed in the newborn and continue into adolescence and possibly even further into adulthood [22]. The phylogenetically youngest brain regions, such as frontal white matter, are the last to myelinate, whereby a posterior-to-anterior gradient in terms of myelination is present [23]. Other cells in the white matter include astrocytes and microglia.

White matter tracts may be categorized into 3 different types based on their topological projection. Association fibers are usually long-range fibers, which connect intra-hemispheric areas and includes the superior and inferior longitudinal fasciculus, arcuate and uncinate fasciculus and the cingulum. A subset of association tracts are called U-fibers which are short tracts running immediately subjacent to the cortex and connects neighbouring gyri. Projection tracts runs perpendicular to association fibers in an ascending-descending or descending-ascending manner. These include tracts between the cortex and the spinal cord. Lastly, commissural tracts connect the two hemispheres, and includes the largest fiber tract in the human brain, namely the CC. The anatomy and function of the CC will be the focus of a subsequent section. Other notable commissural tracts are the fornix commissure sometimes also referred to as the hippocampal commissure, and the anterior and posterior commissure [24].

As in other areas of anatomy, white matter anatomy is multi-faceted and multi-hierarchical. This has led to divisions of the white matter based not only on the aforementioned tracts. For example the white matter surrounding the lateral ventricles may be referred to as the periventricular white matter whereas white matter subjacent to the cortex may be referred to as subcortical white matter. However, no clear boundary between these two areas have been defined, and a distinction may make more sense in the context of the presumed difference in susceptibility to vascular lesions.

The blood supply to the white matter may be separated into zones: that to the subcortical white matter containing U-fibers, and that to the deep, periventricular white matter. From the leptomeninges, small arteries penetrating the cortex to the white matter irrigate the subcortical white matter. Furthermore, arteries which do not immediately give off branches when entering the white matter, penetrate deeper towards the periventricular white matter. Here, the arteries branch off for irrigation of the deep white matter, but also gives rise to branches coursing back towards the subcortical white matter. This provides the subcortical white matter with redundancy in its blood supply, and may be the source of the relative resistance of the U-fibers to ischemic damage [25,26].

Several different pathologies may more or less selectively affect the white matter such as autoimmune, infectious, metabolic, toxic inflammatory, and vascular disease. With regards to vascular disease affecting the white matter, this includes both large infarcts and hemorrages, which often also involves cortex, but also less striking changes such as lacunar infarcts, microbleeds and white matter lesions [27]. Regarding white matter lesions, also called leukoaraiosis, these changes may be observed on MRI as white matter hyperintensities or on CT as hypodense areas [28], and are associated with hypertension, diabetes and other vascular risk factors, and is thought to result from small vessel disease [26,29]. However, the most prominent risk factor is aging, and white matter hyperintensities are prevalent in elderly subjects [29]. In the context of the Leukoaraiosis And DiSability (LADIS) study, these changes have been referred to as age-related white matter changes (ARWMC).

AGING AND DEMENTIA

Within the last decades, the number of persons living to old age (arbitrarily defined as age over 65 years) has increased dramatically, and will continue to do so in the next decades. Since the most significant risk factor for dementia is age (i.e. increasing age increases one’s risk), one consequence of the aging popula-
tion is an increase in the number of persons living with dementia [30]. However, dementia is not a natural consequence of aging, but represents the presence of pathological neurodegeneration which leads to cognitive decline and loss of functional abilities which sharply deviates from what may be expected at a given age. AD is the most prevalent neurodegenerative dementia, roughly responsible for 40-50% of dementia cases [31]. Clinically, patients with AD often present with early and prominent episodic memory deficits, although deficits in other cognitive domains may be present. Steady progression with increasing cognitive impairment which will include more and more cognitive domains is a hallmark feature. The fundamental understanding of the neuropathological changes leading to AD have been greatly advanced within the last 20 years, highlighting deposition of beta-amyloid and atrophy of grey matter, the most salient of which is hippocampal atrophy, as central components in the pathophysiology of the disease [32]. However, despite the growing knowledge of disease mechanisms, disease-modifying treatments are lacking, and there is still a great need for further diagnostic tools. Moreover, several aspects of the pathophysiology remains to be elucidated, including the role of white matter atrophy. Other neurodegenerative diseases, which are prevalent causes of dementia, include vascular dementia (VaD) and fronto-temporal dementia (FTD). VaD is characterised by vascular changes, either larger strategic infarcts or smaller vascular changes such as lacunar infarcts and white matter lesions [33].

As highlighted above, dementia represents a pathological decline in cognitive function, distinctive from the normal aging process. However, normal aging is also associated with decline in cognitive function (possibly mostly so for executive and processing speed) and atrophy in grey and white matter. In the following sections, the age-associated CC changes and changes in the CC in dementia will be reviewed.

STRUCTURE AND BASIC FUNCTION OF THE CC

The CC is a massive white matter commisural tract located in the middle of the cerebrum. On the mid-sagittal slice of an MRI, the structure is reminiscent of a C-shape with the convexity dorsally. Anteriorly, the CC forms a hook whereby it doubles back on itself for a few milliones. Posteriorly, the CC bends at a lesser angle while forming a bulbosity. The CC may be divided into 4 anatomical areas, although no clear definitions exist to delineate them. From anterior to posterior they are rostrum, genu, midbody and splenium [34]. (See figure 1).

Callosal axons arise from neurons with their soma in cortical layer V and to a lesser extent layers II,III and VI [35]. The projections primarily connect contralateral homotropic cortical areas (i.e. left frontal cortical areas with right frontal cortical area), but there is evidence to suggest that heterotropic projecting neurons exist as well as neurons with dual projections [36,37] and projections to subcortical structures, although the latter has only convincingly been established in animals [35].

The CC has a rich blood supply with frequent anastomoses, possibly mitigating the incidence of isolated callosal infarcts [38]. From the anterior communicating artery the subcallosal and the median callosal artery approaches the CC from below and curves around the anterior CC continuing on the dorsal aspect of the CC. In most subjects, one of the two arteries is the dominant artery and irrigates the anterior and, in some instances, also the middle of the CC. The pericallosal artery, also arising from the anterior communicating artery, gives off several named branches all supplying the CC. These form the pericallosal pial plexus. The posterior CC is supplied by the posterior pericallosal artery from the posterior cerebral artery [25,39].

Since the CC is the largest commisural tract with approximately 200 million axons [40], there is general consensus in the literature, that the most basal function of the CC is interhemispheric communication, as evidenced by studies linking different measures of interhemispheric transfer of information to anatomic properties of the CC [41,42]. However, the neuronal actions by which this is achieved and what exactly this large capability for information transfer may be necessary for, remains to some degree a matter of speculation. Two models have been suggested by which the CC may facilitate interhemispheric transfer.

The first model suggests that the CC is a conduit through...
which the two hemispheres exchange and coordinate information (excitatory model) [41], e.g. sensory information, and the second model, that the CC enables one hemisphere to modulate and inhibit the function of the contralateral hemisphere (inhibitory model) [43] in order to limit processing to one hemisphere when appropriate. Such models may be relevant to both cognitive as well as motor function. The suggested models of basal callosal function are not mutually exclusive. Indeed, both large myelinated axons, which conduct information at a high speed, and small unmyelinated axons for slower modulatory effects [40,44] are present in the CC, and may be seen as structural cellular properties supporting such a dual function of the CC [45,46]. Furthermore, results from studies applying electrophysiological methods to the study of the CC have found that the structure may both inhibit and facilitate activity in the contralateral hemisphere. Moreover, the speed with which information transfer occurs, seem to be dependent on the directionality of the transfer, with the fastest transfer when information is transferred from the non-dominant to the dominant [47]. The laterisation of some cerebral functions have been suggested to be reliant on callosal function, and in this context both an inhibitory and excitatory function of the CC may be a prerequisite. That is, inhibition of the hemisphere not participating, and transfer of information, such as sensory, in situations where information is perceived by one hemisphere and processed in the contralateral [48].

The distribution of axons of varying diameter in the CC may further help inform of the structure-function relationship in the CC. Axons of varying diameters are not randomly distributed across the CC. Rather, they are roughly distributed according to the cortical areas callosal neurons project to [40]. In the anterior CC, axons projecting to areas in prefrontal cortex are of small diameter. In contrast, in the midbody of the CC, which projects to motor and somatosensory cortex, large-diameter axons are found. In the posterior splenium, larger diameter axons are also found, possibly connecting areas of visual cortex, whereas in the anterior part of the splenium and posterior midbody, smaller-diameter axons may connect to association cortex in the parietal, temporal and occipital cortex [44,49,50]. This topography of axons have led to the suggestion that the CC may best be conceptualized as a collection of different tracts with unique structural properties and function, rather than one single tract [51]. It should be noted that the cortical projections of the CC remains an area of continued investigation. To that end, advances in diffusion-weighted MRI (DW-MRI) has contributed greatly. In DW-MRI the MR signal is sensitized to diffusion of water. Since the diffusion of water molecules will be affected by barriers such as myelin and cell-walls of neurons, it is possible to characterize and quantify certain aspects of white matter microstructure. Moreover, it is possible to reconstruct tracts in the white matter in 3-D, so-called tractography, which has been used to explore callosal projections to the cortex [52]. Studies applying these methods to chart the callosal projections have come to somewhat divergent results. However, some results are noteworthy, and consistently reported across studies. Firstly, most studies find the largest cortical area to send projections through the CC is the frontal cortex, covering approximately the anterior 1/3 of the CC. Secondly, temporal and parietal cortex seemingly has relatively few connections through the CC. Although not an unexpected finding, since mesial temporal lobe structures are connected through the hippocampal commissure, rather than the CC, this finding may also be confounded by the failure of tractography to identify laterally projecting callosal fibers [36]. Tractography algorithms tend to fail in areas with crossing fiber populations such as the area where laterally projecting callosal fibers pass through. Thirdly, some overlap in the CC with respect to projections exists so that for example neurons from frontal and motor cortex intermingle [49,50,53,54]. These results, however should be interpreted with caution since they lack confirmation from postmortem neuroanatomical investigations, and since DW-MRI and tractography as a method needs further neuroanatomical validation [52].

COGNITIVE AND MOTOR FUNCTIONS RELATED TO THE CC

Several different strategies have been employed in the effort to delineate the cognitive and motor functions reliant on intact callosal connections. One such strategy is the study of so-called split-brain patients, who have undergone transection of the CC in order to inhibit the interhemispheric spread of epileptic activity. Some patients may show severe neurological problems following the surgery, whereas others have very subtle deficits, evident only when patients are subjected to tests specifically designed to evoke them. These include deficits in visuo-spatial processing, language comprehension, self-recognition, abstract reasoning (comprehension of causality), moral reasoning and learning new bimanual tasks [42,55–57]. Moreover, experiments with patients undergoing partial sectioning of the CC have indicated, as one would expect, that different parts of the CC is involved in different functions, with the posterior CC being more involved in visual processing, and the anterior involved in higher cognitive functions [57,58]. Other experiments in patients with isolated vascular or neoplastic lesion as well as studies in patients with congenital abnormalities of the CC have further indicated that many other cognitive functions may be reliant on the CC. This includes attention [59,60], processing speed, category fluency [61], verbal memory [55], apraxia [57], alexia without agraphia [62], dyslexia and others [57,63,64]. Regarding callosal motor functions, focus has been on bimanual tasks which require coordination of the movements of the limbs. Patients who have undergone callosotomy and acallosal subjects display deficits in bimanual tasks, possibly more pronounced with learning new bimanual tasks [64]. Structural properties of the CC have also been shown to predict how well healthy subjects acquire a new bimanual task [64–68]. Furthermore, there is evidence that children showing isolated agenesis of the CC reach motor learning milestones later, compared to healthy children, primarily when it comes to skills requiring bimanual skills, such as riding a bicycle [69]. Another deficit which may impair motor function is a reduced ability for the transfer of sensory input between hemispheres. For example, callosotomized patients may show deficits in tasks in which sensory stimuli acts as a cue for eliciting a certain motor response, when this stimulus is presented to the contralateral hemisphere from which the motor response should be evoked [42].

However, several major caveats must be highlighted regarding these strategies of investigation. The data mostly arises from case studies of a small number of individual patients, sometimes with the same patient being teated again and again over several decades [70]. This method is very useful especially for hypothesis generation, but is sensitive to individual variations and often
precludes statistical testing. Furthermore, patients with callosal lesions, congenital abnormalities of the CC and callosotomized patients may have pathology in other brain structures, which may confound the findings. Lastly, it is important to highlight that the modes of lesion in these patients are fundamentally different than the atrophy and vascular lesions associated with dementia and old age, and therefore the cognitive deficits associated with CC atrophy may likewise differ fundamentally. For example, callosal atrophy may be a consequence of cortical atrophy which may lead to other deficits compared to those caused by direct damage to the CC.

Overall, the cognitive deficits reported to result from callosal damage are heterogeneous, and may be influenced by confounding factors as already highlighted. However, if interpreted in line with the “connectionist” brain model presented earlier in the thesis, it may be a result of the involvement of the CC in several different functional networks. Therefore, pathological changes in the CC may very well lead to very diverse cognitive impairments.

IMAGING OF THE CC

The fundamental assumption behind any investigation of structure-function relationships is that the way in which the structure is quantified relates to some biological phenomenon which may impact the function of the structure. For the CC, measurement of the mid-sagittal area has been the most widely applied method for quantification in imaging research. At this point in the brain, the callosal fiber are densely packed as opposed to more laterally, and therefore offers the most salient point for quantification. A few studies have examined whether the area of the CC correlates with fiber density. Abotiz et al [40,71], investigated a large sample, and found that there was a correlation in areas with predominantly smaller diameter axons (< 3 μm). This may be due to a higher variability of the fiber diameter in areas of the CC with larger diameter axons. Two other studies found a clear positive correlation between CC area and fiber density both for areas with small and large diameter axons and areas where different populations of axon diameter were mixed [72,73], indicating that CC area is principally influenced by axon number and not by axon diameter in aging. A similar relationship has been reported in another study [44] and also in the Macaque monkey [74]. However, it is possible that other factors may contribute, such as demyelination and change in the density of axons, but this remains speculative. Since the CC is devoid of macroanatomical landmarks which may be used to subdivide the CC, neuroimaging studies have applied different geometric partitioning schemes. The most widely used partitioning scheme is based on Witelsons partitioning scheme [75], which divides the CC into 5 subregions either with radial or vertical dividers (Figure 1). The Witelsons partitioning scheme is based on the assumed projections of callosal axons to the cortical lobes. Other partitioning schemes with fewer or more subregions have been used [76–79]. Furthermore, the application of DW-MRI and tractography to CC anatomy has indicated that other partitioning schemes may better capture the projections of callosal neurons to cortical areas, but these results need postmortem validation.

Other methodologies have been applied for measuring the size of the CC. These include callosal height [80], width [81] and bending angle [82]. Furthermore, DW-MRI has also been used in several studies [19,83,84] as has other MRI derived measures [85,86] which assesses other aspects of the CC such as cellular microstructure.

NEUROPATHOLOGICAL CHANGES LEADING TO CC ATROPHY

The underlying mechanisms of CC atrophy in aging and dementia in not yet fully elucidated. However, two mechanisms are often highlighted in the literature. These are Wallerian degeneration due to cortical atrophy and damage to callosal fibers caused by white matter lesions, such as ARWMC. Findings so far seem to indicate that both mechanisms may be associated with CC atrophy in different populations [78,87–89]. Other mechanisms may also be involved in CC atrophy. For example, transsynaptic degeneration in which neurons degenerate due to lesions of neurons which synapse on them, may play a role, as may diaschisis [27]. Furthermore, processes which may not lead to total loss of the axon, such as demyelination may also be an important factor in CC atrophy. It should also be highlighted, that pathological processes which are not detectable by structural MRI may lead to changes in callosal function. This includes slowed or absent conduction of action potentials and asynchronous activity in neural networks which the CC is involved in. Such pathological changes may be detectable using neuropsychological examination [42], eletro-neurophysiological measurement [41], transcranial magnetic stimulation [47] and fMRI [90].

PREVIOUS FINDINGS IN AGING AND DEMENTIA

Structural changes of the CC in old age have been investigated in several cross-sectional MRI studies. A decline in size and microstructural integrity in anterior parts of the CC is often reported as being the most predominant finding [83,91–94], and is corroborated by postmortem findings [73,95].

Several cross-sectional studies have found an association between age-related CC atrophy and global cognitive function [96–98]. Jokinen et al [99] investigated more specific cognitive domains in the LADIS cohort and found anterior CC atrophy to be associated with slower psychomotor speed and poorer performance on tests of executive function. Other studies have found similar associations between anterior CC size and measures of processing speed and executive function as well as working memory [100–102]. For processing speed an association to changes in microstructure measured by DW-MRI has been reported [103].

Another interesting observation which may be relevant to the role of the CC in relation to cognitive aging, comes from functional MRI studies. It has been observed that tasks which elicit lateralized brain activation in young persons, may elicit brain activation in both hemispheres in elderly persons [104]. This has been termed hemispheric asymmetry reduction in older adults (HAROLD), and is suggested to be a compensatory mechanism of the aging brain relevant for both cognitive [105] and motor functions [106]. It should be noted that the bihemispheric activation in elderly subjects may also be viewed as a failure of the brain to recruit specialized neural mechanisms (the dedifferentiation hypothesis), possibly due to degenerative changes in the CC [107], or that the right hemisphere is more susceptible to age-related decline [108]. However, compensatory mechanisms and detrimental effects may occur simultaneously. e.g. in different parts of the brain, both leading to decreased lateralisation of brain activation [108].

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Whether CC atrophy may be one of the underlying neural mechanisms of motor deficits in the elderly is less well understood. As has already been established, studies have indicated that bimanual motor tasks may be reliant on callosal function [64–68]. However, unilateral motor tasks may also be reliant on callosal functions since these most likely require inhibition of the contralateral cortex, to ensure inhibition of mirror movements. This is supported by the fact that infants and young children show mirror movement, which have been linked to an immature CC [109], and is also observed in syndromes which includes agenesis of the CC [69]. Other indirect evidence for the role of the CC in age-related motor decline comes from observations of decline in bimanual functions with age [110] and observations from fMRI studies that support similar compensatory mechanisms (such as HAROLD) for motor functions in the elderly [37,111,112].

Studies specifically investigating CC atrophy in neurodegenerative diseases have been the subject of only a few studies, and conclusions have been limited by the very heterogeneous patients included. Moreover, this subject has yet to be addressed. First and foremost, there is a lack of studies examining tissue loss over time in an elderly population. Moreover, the association between specific cognitive domains and CC tissue loss remains largely unexplored. Lesional studies have been informative, but as already mentioned, the mechanism of damage may very well influence the observed deficits. The same is applicable to motor functions. Therefore, it remains undetermined whether direct conclusions regarding the effect of CC atrophy and tissue loss in elderly subjects, may be drawn from such lesional studies. Regarding dementia, the specific subregional pattern of CC atrophy in early stages of AD has been the subject of only a few studies, and conclusions have been limited by the very heterogeneous patients included. Moreover, CC tissue loss in elderly subjects who progress to dementia has not been examined.

OBJECTIVES AND HYPOTHESES

The overall objective of the thesis were:

To examine the pattern of CC atrophy in early AD, and its association with progression of global cognitive impairment

To explore the association between CC tissue loss and the development of cognitive and motor impairment in elderly subjects over time

The hypotheses were:

AD is associated with atrophy of the CC even early in the disease course, and CC atrophy is associated with faster cognitive decline

A greater CC tissue loss over the course of 3 years is associated with higher risk of developing cognitive and motor impairment in elderly subjects

BRIEF OVERVIEW OF THE PURPOSE AND DESIGN OF THE STUDIES

Study I

Study I is a cross-sectional case-control study with a group of mild AD patients and a control group consisting of dementia-free subjects with ARWMC. The primary aim of the study was to assess the pattern of CC atrophy in earlier phases of AD. Furthermore, the correlation between CC size and ARWMC, and CC size and MMSE score at 1-year follow-up was assessed.

Study II

Study II is based on data from the LADIS study, a prospective cohort study, and assesses the impact of CC tissue-loss over a 3-year period on progression to dementia, and on global cognitive and motor impairment. The population in the LADIS study were elderly subjects with ARWMC, who were dementia-free at baseline.

Study III

Study III is based on data from the LADIS study, a prospective cohort study and assesses the impact of CC tissue-loss over a 3-year period on impairments in executive and memory functions, and processing speed. The population in the LADIS study were elderly subjects with ARWMC, who were dementia-free at baseline.

MATERIALS AND METHODS

LEUKOARAIOSIS AND DISABILITY IN THE ELDERLY (LADIS) STUDY

The LADIS study is a longitudinal multicenter cohort study with 11 participating centers in 9 European countries (Amsterdam, Copenhagen, Florence, Graz, Gothenburg, Helsinki, Huddinge, Lisbon, Paris, Mannheim, Newcastle-upon-Tyne). Design and rationale has been described in detail elsewhere [124]. The study was launched in 2000 with the aim to investigate the impact of ARWMC on the transition to disability in elderly subject (age between 64-85 years). Subjects underwent clinical assessment at baseline followed by yearly assessments for 3 years. MRI were obtained at baseline and at the 3-year follow-up. A total of 639 (f/m: 351/288; Age: Mean 74.1 ; SD ± 5 ) elderly subjects with no preexisting functional disability or dementia (IADL ≤1) were enrolled in the study, all with ARWMC based on MR or CT investigations. Although subjects were without disability, they could
have minor neurological, cognitive, mood or motor complaints, as well as minor neurological problems (including minor cerebrovascular events). Furthermore, some of the subjects were healthy volunteers.

**POPULATIONS**

For study II and III data from baseline and 3-year follow-up clinical assessment including MRIs were obtained from the LADIS study population. For study I, baseline data for LADIS subjects enrolled at the Copenhagen center (Memory Disorders Clinic, Rigshospitalet), as well as a cohort of AD patients recruited independently from the LADIS study at the Copenhagen center, were used.

**Study I**

**AD patients**

Twenty-eight patients referred consecutively to the Memory Disorders Clinic at Rigshospitalet, who met DSM-IV [125] and the McKhann criteria [126] for AD were included in this study. Inclusion criteria were so that the patients recruited were comparable with patients recruited for the LADIS study. Further inclusion criteria were as follows:

- age 64–85 years,
- MMSE >20 (AD patients only)
- informed consent
- Exclusion criteria were as follows:
  - The presence of severe illnesses such as cardiac failure, cancer or other relevant systemic disease which was likely to cause dropout, severe unrelated neurological diseases, leukoencephalopathy of nonvascular origin (immunological, demyelinating, metabolic, toxic, infectious, other) or severe psychiatric disorders
  - Inability or refusal to undergo brain MRI.

Dementia-free subjects

As a control group, data from the cohort of LADIS subjects recruited at Copenhagen was used. A total of 50 subjects had MRI at baseline which permitted quantification of the CC and were subsequently included in the study.

**Study II & III**

Of the 639 subjects enrolled in the LADIS study, 73 dropouts and 43 deaths had occurred at 3-year follow-up. Furthermore, at follow-up 30 patients refused to undergo MRI and 1 center was unable to obtain funding for follow-up MRI for 47 patients, leaving a total of 446 subjects. Only subjects for whom baseline and 3-year follow-up MRIs of sufficient quality were available could be included, leaving a total of 328 subjects. In this cohort it has previously been reported that patients with baseline and follow-up scans were younger, had longer education and higher MMSE score at baseline than subjects with baseline only [127]. However, there were no differences in these variables between those subjects with a follow-up MRI which enabled quantification of the CC and those with a follow-up MRI which did not (p<0.05).

Of the 328 subjects, 9 subjects were excluded from study II and 75 subjects for study III due to missing imaging variables leaving 253 subjects for study III. Furthermore, a number of the remaining subjects had missing data for the clinical data (dementia n=9; motor scores: SLS n=138; SPPB n=110; walking speed n=20). For the analysis of self-percieved memory impairments, subjects were excluded for analysis if the participant had developed dementia at 3-year follow-up or had missing data with regards to dementia status (n=44). See Figure 2 and 3 for flow-charts of the populations in study II and III.

**MRI**

**MRI sequences and scanner – Study I**

A 1.5-T scanner (Vision, Siemens) was used to acquire the scans for study I. The MRI protocol consisted of 3-D sagittal T1-weighted magnetized prepared rapid gradient echo (MPRAGE) images (dementia free subjects: TE = 7 ms; TR = 13.5 ms; FA = 15°; voxel size = 0.98 * 0.98 * 1 mm; FOV = 250 mm; AD patients: TE = 4.4 ms; TR = 11 ms; FA = 8°; voxel size = 0.98 * 0.98 * 1 mm; FOV = 250 mm) and fluid attenuated inversion recovery (FLAIR) images (dementia free subjects: TE = 110 ms; TR = 9000 ms; TI = 2400 ms; FOV = 250 mm; slice thickness = 5 mm; AD patients: TE = 110 ms; TR = 9000 ms; TI = 2500 ms; FOV = 250 mm; slice thickness = 5 mm).

**MRI sequences and scanners – Study II & III**

Baseline and follow-up MRIs were conducted according to the same standard scan protocol. For baseline scans, 10 centers used 1.5-tesla scanners, and 1 center 0.5 T, whereas for follow-up 3 centers had acquired new scanners, so only 1.5-tesla scanners were used. To ensure scanner sequence homogeneity across centers, phantoms were used. The MRI protocol included the following sequences: MPRAGE; (scan parameters: coronal or sagittal plane, TE: 2–7 ms, TR: 9–26 ms, flip angle: 15–30°, voxel size 1 * 1 * 1–1.5 mm 3 ), T2-weighted fast spin echo (scan parameters: axial plane, TE: 100–130 ms, TR: 4,000–6,600 ms, voxel size 1 * 1 * 5 mm 3 , 19–31 slices), and FLAIR (scan parameters: axial plane, TE: 100–160 ms, TR: 4,000–6,600 ms, voxel size 1 * 1 * 5 mm 3 , 19–31 slices).
Assessment of the CC mid-sagittal area

For assessment of CC, MPRAGE images were reoriented to standard Montreal Neurological Institute template orientation, using a 6-parameter rigid transformation SPM5 software package (http://www.fil.ion.ucl.ac.uk/spm/spm5.html). For study II and III baseline MPRAGE images were coregistered to follow-up MPRAGE images at this point.

Next, the CC was localized automatically on the midsagittal section of the MPRAGE dataset using learning-based active appearance models (AAMs) [128]. In short, AAMs parameterize object variability from a manually specified training set, and derive a constrained deformation basis enabling the localization of objects similar to the training set. The algorithm is based on a computational framework described elsewhere. The training set consisted of 569 subjects from a cohort of similar ages and the resulting AAMs delineated the CCs in the entire data set. A trained reviewer unaware of the clinical status subsequently corrected for any inaccuracies. Finally, the CC was automatically outlined and divided into 5 subregions using a modification of the Witelson partitioning scheme (Figure 1). This rotates the CC into a coordinate system in which the X-axis is parallel to the longest axis (the first principle axis) of the structure, and divides it into fractions containing the superior 95% and the inferior 5% of the total area; the Y-axis of the coordinate system passes through the center of gravity. Radial dividers emanating from the origin with equal angular spacing were used to subdivide the CC into rostrum and genu (CC1), rostral body (CC2), midbody (CC3), isthmus (CC4) and splenium (CC5). The area of each segment was calculated automatically. The method used to outline the CC is independent of voxel coordinates, and therefore has subvoxel resolution, and inherently corrects for partial volume effects. The areas of each segment were calculated automatically and adjusted for head size, i.e. skull size, by registration (12-parameter affine) of the MPRAGE to a standard brain template (Montreal Neurological Institute template) from which scaling parameters along the y- and z-axes were derived. Each CC area was subsequently multiplied by the individual parameters.

Assessment of progression of ARWMC (Study II & III)

Visual rating of ARWMC progression from baseline to 3-year follow-up was done using the modified Rotterdam Progression Scale [129]. The scale scores progression in 9 brain areas (no progression = 0, progression = 1) yielding a score between 0 and 9 for each subject. Scoring of scans was carried out in a side-by-side manner by a single rater blinded to clinical status. To identify lacunes, fluid attenuation inversion recovery, MPRAGE and T2 images were used.

Assessment of ARWMC volume (Study III)

ARWMC volume was analyzed at baseline on the axial FLAIR images by a single rater using a semi-automated method [130]. The lesions were marked by the rater, and borders were set by using local thresholding on each slice. This was followed by auto-
matized calculation of the total volume of all delineated ARWMC.

Assessment of ARWMC using the Fazekas scale (Study I)

For ARWMC assessment, visual rating on the FLAIR images was performed using the modified Fazekas scale [28] which categorizes the ARWMC into 3 severity classes (mild/moderate/severe). All ratings were performed by an experienced rater (E.G.) blinded to the clinical data.

Assessment of medial temporal lobe atrophy (Study II & III)

Medial temporal lobe atrophy (MTA) was visually assessed at baseline according to the MTA scale [131]. Assessment was done on the coronal T1-weighted sequence with a possible score of 0–4: 0 = no atrophy; 1 = widening of the choroid fissure; 2 = widening of the choroid fissure and temporal horn; 3 = widening of the choroid fissure and temporal horn and diminishing height of the hippocampus; 4 = severe atrophy. The mean of the left and the right scores was used.

Assessment of whole brain atrophy (Study III)

Brain atrophy was assessed at baseline with a template-based visual rating scale ranging from 1 (no atrophy) to 8 (severe atrophy) separately on ventricular and sulcal areas as validated before in the LADIS study. The sum of the two ratings was used as a global measure of atrophy.

ASSESSMENT OF GLOBAL COGNITIVE FUNCTIONS

MMSE

The MMSE is a 30-item cognitive screening instrument, and was used to assess global cognitive function [132]. For study II and III, MMSE scores were obtained at baseline and 3-year follow-up. For study I MMSE scores were obtained at baseline and after 1-year follow-up.

ASSESSMENT OF SPECIFIC COGNITIVE DOMAINS

A total of 3 specific cognitive domains were assessed in study II at baseline and 3-year follow-up. These included the following domains: Speed and motor control (processing speed), executive function and memory functions. A battery of tests were used for this purpose, and compound scores for each domain were calculated based upon the score from these tests. Calculation of compound scores will be reported in a following section of Statistical analysis.

Vascular Dementia Assessment Scale-cognitive subscale

The Vascular Dementia Assessment Scale-cognitive subscale (VADAS) is an extension of the ADAS-cog designed for patients with cognitive impairment presumed to be on a vascular basis [133,134]. The additions included in the VADAS are as follows: delayed word recall, symbol digit modalities test, digit span, maze, digit cancellation, and verbal fluency.

Stroop test

The Stroop test is widely accepted as a measure of certain aspects of executive functions [135]. The test consists of 3 sheets. The first sheet is color word printed in black (subjects are asked to read out loud the color word), the second is colored X’s (subjects are asked to read out loud the colors), and the third is color words printed in incongruous colored ink (subjects are asked to read out loud the color of the ink).

Trail making test

The Trail making test is a measure of processing speed, sequencing, mental flexibility and visual–motor skills [136]. It consists of a part A (subjects are asked to connect sequential numbers) and a part B (subjects are asked to connect sequential numbers and letters interleaved starting with 1 followed by A).

Processing speed

For the assessment of processing speed, the time to complete part A of the Trail making test was used. Furthermore, from the VADAS, the number of correct responses for the digit cancellation and the time to complete the maze, was used.

Executive function

For the assessment of executive functions the subtraction score for the Trail making test (B–A) and the subtraction score for the Stroop test (color incompatible – color neutral) was used. From VADAS verbal fluency (number of animal names generated in 60 s) and the symbol digit modalities tests (number of correct responses) was used.

Memory functions

For the assessment of memory functions the following subtasks form the VADAS were used: word recall, delayed recall, word recognition, and digit span subtasks.

ASSESSMENT OF MOTOR FUNCTIONS

The short physical performance battery (SPPB) is primarily used to assess lower limb strength and balance. It consists of 3 subtasks, which are given a score between 0-4 each based on performance on each item. The total SPPB hence ranges between 0-12. The subtasks were: Standing balance: Ability to balance one one leg was the outcome measure used. A maximum limit of 30 s was applied, and a SLS time ≤ 15 s was considered pathological.

Single leg stance time

Single leg stance time (SLS) is a measure of balance, and poorer performance is related to the risk of falls [139]. SLS was measured by asking participants to stand on one leg with their hands on their hips. This was carried out a total of 4 times (twice for each leg). The time participants were able to remain balanced on one leg was the outcome measure used. A maximum limit of 30 s was applied, and a SLS time ≤ 15 s was considered pathological.

Gait speed: Participants were timed from a standing start and asked to walk at their normal pace over a 4m course. The fastest of two trials was used. Lower extremity strength: With their arms crossed in front of their chest, participants were asked rise from a
chair as quickly as they could five times. A score ≤ 10 on the SPPB was considered pathological.

**Walking speed**
Walking speed was calculated using timed walks on an 8-meter course, which participants were asked to complete twice at their normal walking speed. The fastest time was used to calculate walking speed. A score ≤ 1.2 m/s was considered pathological.

**DEMENTIA DIAGNOSES**
For the identification of subjects with dementia in study I and III the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria for dementia, were applied. For diagnosis of subtypes of dementia in study III and diagnosis of patients with AD in study I, the following diagnostic criteria were used:
- Alzheimer’s disease
  - For the diagnosis of AD, the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [126] were used.
- Vascular dementia
  - For the diagnosis of subcortical VaD, the criteria of Erkinjuntti et al. [140] and the National Institute of Neurological Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINDS-AIREN) criteria for VaD [33] were applied.
- Fronto-temporal dementia
  - For the diagnosis of frontotemporal dementia, the criteria of McKhann et al. [141] were applied.

**STATISTICAL ANALYSIS**
Annual percent change in CC areas was calculated using the following formula: \[
\text{[(CCfu) – (CCb)]/(CCb * ISI)] * 100%},
\]
where CCfu = CC area at follow-up, CCb = CC area at baseline and ISI = interscan interval.

Different statistical approaches were applied for the 3 studies. In general, in study I and II logistic regression analyses were applied to assess the association between dichotomous outcomes. For study I Spearman’s rank correlation was used to assess associations between CC size and MMSE and ARWMC as well as ARWMC and MMSE. For study II, multiple linear regression analysis was used to assess an association between CC tissue loss and MMSE. In study III associations of CC change to change in cognitive performance was analyzed with hierarchical linear regression models.

For regression analyses in study II and III, these were adjusted for relevant clinical, demographic and imaging covariates. Prior to analysis, data was assessed for missing values, outliers and non-normality, and if necessary the data were transformed. Statistical significance was set at \( p < 0.05 \).

**RESULTS**
The results presented in the following represent the main findings of the study and addresses the main objectives and hypotheses presented earlier in the thesis. For full details please see the Appendix. In general, results are reported as mean (SD) if not otherwise stated.

**STUDY I**
Baseline demographic and clinical summary statistics are found in Table 1.

**TABLE 1: BASELINE DEMOGRAPHICS, IMAGING AND CLINICAL CHARACTERISTICS**

<table>
<thead>
<tr>
<th></th>
<th>Mild AD (n=28)</th>
<th>Dementia-free subjects (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female/male)</td>
<td>21/7</td>
<td>28/22</td>
</tr>
<tr>
<td>ARWMC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>15 (54)</td>
<td>30 (60)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (25)</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Severe</td>
<td>6 (21)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>76.0 (±6.5)</td>
<td>74.1 (±5.2)</td>
</tr>
<tr>
<td>MMSE (mean ±SD)</td>
<td>24.7 (±1.9)***</td>
<td>28.4 (±1.3)</td>
</tr>
</tbody>
</table>

For ARWMC number of subjects per category is shown with percentage of subjects in paranthesis.
- AD= Alzheimer’s disease
- ARWMC=Age-related white matter changes
- SD= standard deviation
- MMSE=Mini-mental status examination

*** \( p < 0.001 \) between groups.

**What is the subregional pattern of CC atrophy in mild AD**
We found that total CC size (\( t = 1.64, p = 0.05 \)), CC3 (\( t = 1.68, p < 0.05 \)) and CC5 (\( t = 2.79, p < 0.01 \)) was significantly smaller in AD patients when compared to controls. When controlling for age, gender and ARWMC, only CC5 (\( p < 0.05; OR = 0.81; range = 0.65–0.99 \)) remained significant (Table 2).

**TABLE 2: AREA OF CC AND CC SUBREGIONS**

<table>
<thead>
<tr>
<th></th>
<th>Mild AD (n = 28), mm²</th>
<th>Dementia-free subjects (n = 50), mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Total CC</td>
<td>426.1a</td>
<td>18.7</td>
</tr>
<tr>
<td>CC1</td>
<td>132.3</td>
<td>5.7</td>
</tr>
<tr>
<td>CC2</td>
<td>58.2</td>
<td>3.1</td>
</tr>
<tr>
<td>CC3</td>
<td>56.2b</td>
<td>2.9</td>
</tr>
<tr>
<td>CC4</td>
<td>62.4</td>
<td>2.7</td>
</tr>
<tr>
<td>CC5</td>
<td>113.8abc</td>
<td>6.0</td>
</tr>
</tbody>
</table>

\( a \) \( p < 0.01 \) in univariable analysis.
\( b \) \( p < 0.05 \) in multivariable analysis.
SE= Standard error.

**Is CC size correlated with ARWMC?**
We did not find any correlations between ARWMC score and total CC size, or size of any subregions in AD patients or in controls.
Is CC size correlated with MMSE at baseline or change in MMSE after one year?

Annual change in MMSE score in AD patients was –1.48 (±2.2) (p<0.05 for univariable analysis for change within group) and –0.38 (±1.7) (p>0.05) for controls. In AD patients a correlation was found between total CC size and annual change in MMSE from baseline to 1-year follow-up (rs = 0.42; p < 0.05). Furthermore, for subregions a correlation was found for CC2 (rs = 0.62; p < 0.01) and CC5 (rs = 0.52; p < 0.01) and annual change in MMSE (Figure 4). We did not find any correlation between CC size and baseline MMSE. Similarly, we did not find any correlation between baseline MMSE and total CC or CC subregional size or annual change in MMSE and total CC or CC subregional size in controls.

FIGURE 4: TOTAL CC AND CC SUBREGION AREA AND CHANGE IN MMSE AFTER ONE YEAR

The graph on the left shows scatter-plot and linear prediction of total CC area and change in MMSE. The graph on the right shows the same for CC2 and CC5.

STUDY II

Baseline demographic, clinical and imaging characteristics are found in Table 3 and data for 3-year follow-up in Table 4. For logistic regression analysis the following covariates were entered: Baseline MMSE, age, gender, modified Rotterdam progression scale score, MTA score, incident lacunes, and presence of diabetes.

Is dementia at 3-year follow-up associated with annual CC tissue loss

Forty-four (13.4%) patients had received a diagnosis of dementia at the 3-year follow-up. Of these, 13 patients were diagnosed as having AD, 30 patients VaD and 1 frontotemporal dementia. Due to the relatively small numbers, statistical analysis was conducted on the total group of converters, not on subtypes. There was a significant difference in annual CC tissue loss in CC5 between subjects who had not progressed to dementia at 3-year follow-up compared to subjects who had progressed to dementia by 3-year follow-up (Odds ratio (OR): 0.81; 95% confidence interval (CI): 0.69–0.96; p < 0.05). This indicated that subjects who had developed dementia had more severe tissue loss in CC5.
Is global cognitive function at 3-year follow-up associated with annual CC tissue loss?

We found that tissue loss in CC5 was independently associated with MMSE score at 3-year follow-up (β = 0.21; 95% CI: 0.05–0.38; p < 0.05). The association indicated that more severe atrophy in CC5 was associated with a lower MMSE score at 3-year follow-up.

Is motor function at 3-year follow-up associated with annual CC tissue loss?

For SLS, logistic regression analysis revealed a predictive value of the total CC (OR = 0.70; 95% CI: 0.55–0.88; p < 0.01), CC1 (OR = 0.80; 95% CI: 0.68–0.95; p < 0.01), CC3 (OR = 0.86; 95% CI: 0.76–0.97; p < 0.05) and CC5 (OR = 0.72; 95% CI: 0.59–0.88; p < 0.01). There was no significant predictive value of CC tissue loss on other motor test scores (SPPB, walking speed), or on the history of falls.

STUDY III

Baseline demographic and clinical summary statistics are found in Table 5. For analysis in this study, covariates were entered in a step-wise manner. First, in one model age, education, sex, and baseline cognitive performance (composite scores for the three domains) were entered. In the second model baseline ARWMC volume, baseline brain atrophy rating, progression of ARWMC were also entered.

TABLE 5: BASELINE DEMOGRAPHICS, IMAGING AND CLINICAL CHARACTERISTICS FOR THE COHORT IN STUDY III (N=253)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean (±SD))</td>
<td>73.7 (5.0)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>130 (51) / 123 (49)</td>
</tr>
<tr>
<td>Education (mean (±SD))</td>
<td>10.3 (3.8)</td>
</tr>
<tr>
<td>Brain atrophy score median (range)</td>
<td>7 (2-14)</td>
</tr>
<tr>
<td>ARWMC volume, mL (mean (±SD))</td>
<td>21.4 (21.9)</td>
</tr>
<tr>
<td>Rotterdam progression scale (median (range))</td>
<td>2 (0-7)</td>
</tr>
</tbody>
</table>

For gender, number of subjects is shown with percentage of subjects in parenthesis.
SD= standard deviation
ARWMC=White matter lesion

Is processing speed associated with annual change in CC size?

In model 1, annual tissue loss in total CC area (β = 0.12, p=0.008), CC1 (β =0.10, p=0.027), and CC3 (β =0.11 (0.016)) was associated with decline in the processing speed compound score, but in the fully adjusted model only total CC and CC3 remained significant.

Is memory functions associated with annual change in CC size?

The association between CC measures and change in memory functions compound scores was non-significant.

Is executive function associated with annual change in CC size?

The association between CC measures and change in executive functions compound scores, were non-significant.

DISCUSSION

The main findings of the studies presented here are that posterior CC atrophy is involved in early AD, and is associated with progression to dementia. Moreover, CC atrophy is associated with cognitive decline in AD patients. In non-demented elderly subjects with ARWMC, CC tissue loss assessed prospectively is associated with cognitive and motor decline.

The following discussion will focus on these major findings. For further discussions of all the findings, please see the papers in the appendix.

CC TISSUE LOSS IN DEMENTIA (STUDY I & II)

In the studies presented here, we investigated several important aspects related to CC atrophy and global cognitive decline, including dementia. We found that posterior CC atrophy was present in early stages of AD, independent of age, gender and ARWMC. A similar pattern was found in study II with annual tissue loss in the posterior CC being associated with progression to dementia over a 3-year follow-up period. The longitudinal findings were independent of baseline MMSE, age, gender, progression of ARWMC, baseline medial temporal lobe atrophy, incident lacunes, and presence of diabetes.

Our findings in AD patients is consistent with previous studies in mild to moderate AD in which involvement of the posterior CC, i.e. the splenium, either isloated [117,119] or concomitant with other subregions [118,120], has been reported. However, some studies have not found evidence of posterior CC atrophy, and indeed have found the CC unaffected in mild to moderate AD [79,142]. To our knowledge only one study has examined the CC in severe AD, and reported atrophy of anterior and posterior CC as well as the anterior part of the midbody [118]. In patients with Mild Cognitive Impairment, a condition which is thought to represent a pre-dementia stage of AD, the majority of studies [118,119,143,144], but not all [145–147], have found the CC to be unaffected. Although there is a scarcity of studies using homogenous patient populations and results are not consistent, the findings seem to indicate that CC atrophy is absent in the pre-dementia stage, and first appears in mild AD, in the posterior CC. As the disease progresses, anterior CC is affected, and later the midbody. The present findings support such a model at least in regards to early stages of AD. Our population represented a comparatively homogenous group of AD patients regarding disease stage (MMSE>20) compared to other studies examining the CC, and therefore contributes with important data for the elucidation of CC atrophy in AD. Although we did not directly test this in the present study, such a model would indicate that CC atrophy is a relatively late occurring neuropathological event compared to e.g. hippocampal atrophy and deposition of beta-amyloid.

Further elucidation of the temporal evolution of CC atrophy is encumbered by the dearth of longitudinal studies. To our knowledge only one longitudinal study conducted in a small and heterogeneous, with regards to disease stage, group of patients with AD has been conducted [78]. In this study a higher rate, relative to healthy elderly controls, of tissue loss was found in anterior and posterior CC. We were able to examine whether annual tissue loss was associated with incident dementia in a cohort of elderly subjects with ARWMC who were free of dementia at baseline. We found that annual tissue loss in posterior CC was...
atrophy which, through callosal projections, would give rise to early stages, temporal and parietal cortical areas are affected by dementias, which are presumed to be due to vascular pathology. Only a small number of studies have investigated CC atrophy in VaD. The most consistent finding is atrophy in anterior CC, when compared to healthy controls. When comparing AD and VaD patients there does not seem to be differences in CC atrophy in the anterior CC, indicating that the CC atrophy in VaD is generally less severe than in AD. In contrast, atrophy in posterior CC in AD patients have been reported to be significantly greater compared to VaD, possibly indicating that CC atrophy is more pronounced in AD than VaD.

The functional relevance of CC atrophy in AD remains undetermined. To address this question, it may be of interest to look at possible underlying mechanisms of CC atrophy, and what putative consequences may come from loss of interhemispheric communication. We therefore examined whether CC atrophy was correlated with ARWMC at baseline and MMSE score at baseline and 1-year follow-up in AD patients and non-demented elderly subjects. Several studies have investigated an association between CC atrophy and ARWMCs in AD patients, and have not found support for it. Our study corroborates these findings and extend them by specifically establishing that ARWMC are not correlated with CC atrophy in mild AD, which has not been investigated previously. The finding that CC atrophy is not correlated with ARWMC in AD patients indicates that changes in the white matter is not the principle underlying mechanisms driving CC atrophy in AD. Surprisingly, we did also not find a correlation between CC atrophy and ARWMC in the control group, since a previous study in the LADIS cohort has established an association between CC atrophy and ARWMCs.

A second possible mechanism is that Wallerian degeneration, due to nerve cell body degeneration in the cortex, leads to callosal atrophy in AD. We did not test this hypothesis in any of the present studies but in light of the finding of no association between ARWMCs and CC atrophy in AD patients, it is of interest to discuss other possible underlying mechanisms of action. The proposed temporal course of callosal atrophy, as outlined previously, is consistent with the spread of cortical atrophy in AD. In early stages, temporal and parietal cortical areas are affected by atrophy which, through callosal projections, would give rise to posterior CC atrophy. As the disease progresses, frontal cortex is affected, which may lead to anterior CC atrophy. However, this interpretation should be evaluated in light of the fact that the hippocampal commissure constitutes the only direct interhemispheric connection from the hippocampus, which is the structure most severely and earliest affected by atrophy in AD. Using DW-MRI, Di Paola et al. investigated whether Wallerian degeneration or demyelination may be responsible for CC atrophy in severe AD. Posterior atrophy was found to be due to Wallerian degeneration, whereas anterior CC atrophy was due to breakdown of myelin. Furthermore, posterior CC atrophy correlated with cortical grey matter atrophy, indicating that the observed Wallerian degeneration was due to cortical atrophy. Other studies have found similar associations, although some associations have been less specific with regards to a direct association between callosal subregions and the associated cortical areas.

The difference between subjects in the LADIS cohort who progressed to dementia and non-demented elderly subjects includes visuo-spatial and working memory, language and delayed recall, and found no associations between any test scores and callosal atrophy. Similarly, Chaim et al. also did not find any association with CC size and delayed recall or episodic memory in AD patients. These findings seem to indicate that callosal atrophy in AD patients leads to global cognitive deficits. It may not be unreasonable to speculate that CC atrophy only has a modest detrimental effect even on global cognitive function, and possibly even more so on specific cognitive processes such as episodic memory. One notable exception may be executive function, which may affect global cognitive test scores but not memory and language, and thereby be the underlying cause of the effects on global cognitive function.

CC TISSUE LOSS IN NON-DEMENTED ELDERLY SUBJECTS WITH ARWMC: RELATION TO COGNITION AND MOTOR FUNCTION (STUDY II & III)

In the present studies, we addressed the functional impact of CC tissue loss in a large cohort of elderly dementia-free subjects. We found that callosal tissue loss is associated with decline in global cognitive function, processing speed and SLS but not other areas of cognitive and motor functions. The present study is to our knowledge the first study to examine the association in a longitudinal setting.

Both a decline in processing speed and changes in white matter structure have been reported independently in old age, with the age-span trajectory being an inverted U for both. Furthermore, cross-sectional studies in different populations have indicated that processing speed is reliant on white matter structures, including the CC as found in a previous study using cross-sectional data from the LADIS cohort. Processing speed is often defined as the speed with which different cognitive...
functions may be conducted [159]. It is therefore not surprising that changes in the white matter may affect the processing speed since the insulatory properties of myelin greatly influences the speed with which the action potential may be propagated. We found the association for anterior CC, which is especially susceptible to age-related demyelination [23,27], as well as for the middle of the CC. It could therefore be speculated that myelin break-down resulting in CC tissue loss may be involved in our findings.

We did not find an association between memory and executive function and CC tissue loss. This indicates a divergence in the reliance on the CC between on the one side processing speed, and memory and executive function on the other. As already noted, the hippocampus lacks commissural connections which run through the CC possibly explaining the lack of association to memory. However, this does not totally role out that some parts of the memory process depend on functions mediated through the CC, a supposition which is also warranted with respect to executive function. Indeed, processing speed has been postulated to be underlying other cognitive functions so that a possible association between for example executive function and the CC may in fact be mediated through processing speed [160]. However, is should be restated that in the present study, we did not find an association between CC tissue loss and executive function. Another possible explanation is that processing speed represents a measure which captures callosal functions better as opposed to e.g. executive function.

A salient feature of subcortical ischemic VaD is decline in processing speed [161]. Subcortical ischemic vascular disease SIVD is a white matter disease of vascular origin which is characterised by subtle vascular changes such as white matter changes and lacunar infarcts observable on CT or MRI, and the presence of cognitive and motor deficits. In a population such as the LADIS population the presence of SIVD is of relevance to take into consideration when examining any relationship between brain structures and cognitive deficits [162]. Our findings of an association between anterior CC tissue loss and decline in processing speed was not independent of incident lacunes, baseline ARWMC and progression in ARWMC, whereas that for CC3 and total CC area was. Hence, the association may in part be mediated by the presence of SIVD. This is not surprising since ARWMCs have been shown to be associated with CC atrophy [87].

That an age-related decline in motor functioning occurs, have been established in numerous studies. The underlying mechanisms is most likely multifactorial including peripheral, such as muscle weakness, changes in sensory and proprioceptive nerves, and central mechanisms, such as atrophy of brain regions involved in motor functions, and vascular changes [97,110]. We investigated the association between several different measures of motor function and CC tissue loss, and found that SLS but not SPBB score, walking speed or history of falls was associated with CC tissue loss. Specifically, we found that tissue loss in CC1, CC3 and CC5 was associated with a pathological score on the SLS. SLS is primarily a measure of balance [139], whereas SPBB to a higher degree reflects lower extremity strength [163]. As mentioned earlier, data from callosal agenesis and sectioning of the CC has highlighted especially bimanual tasks as being reliant on an intact CC [42,64]. However, recent studies within the last couple of years combining different imaging modalities, neurological testing and TMS to further elucidate the role of the CC in motor function [37,111,112]. Collectively, the studies indicate that the structural integrity, both in terms of microstructural properties of the tissue as well as CC size, modulate interhemispheric inhibition in relation to simple motor tasks, or in relation to spontaneous motor activity. Interhemispheric inhibition may be important for inhibition of mirror activation of the contralateral limb not involved in a given motor task, which may be especially important when maintaining balance [109]. Wahl et al [37], in a study of interhemispheric inhibition, mapped the somatotopic projections of the hand and foot in the CC, and found that they map approximately to the mid and posterior part of the midbody. This corresponds roughly to CC3 and CC4, and may explain the association between tissue loss in CC3 and decline in SLS in the present study.

Moreover, several studies have indicated that the cognitive contribution to motor tasks increases with age as motor tasks are progressively less reliant on internal automatic movement generation [110]. Together with the increased cognitive contribution is also a more wide-spread activation of cortical areas when complex movements are carried out by elderly persons [106]. This may make certain complex motor functions, such as balance more vulnerable to changes in the anterior CC, which we also found evidence for in the present study.

Previous studies based on the LADIS cohort have investigated the cross-sectional association between different motor scores and CC atrophy [98] as well as the value of baseline CC atrophy in predicting decline on motor function [97]. However, only SPPB and walking speed were investigated in these studies. For both cross-sectional associations and predictive value of baseline CC area, an association was found for SPPB and walking speed, which is contrary to the findings in the present study. This may be due to differences in analysis, since in the present study, we chose to dichotomize the motor scores into pathological or physiological scores, or may reflect underlying biological aspects of the association of the CC and motor functions.

STRENGTHS & LIMITATIONS

The three studies presented in this thesis have different strengths and weaknesses. In the following, these will be presented with sections pertaining to strengths and weaknesses of the individual studies followed by sections which will present those that the studies may share.

In study I we were able to include a relatively homogeneous group of AD patients, which allowed for the exploration of CC atrophy in early stages in AD. The control group were dementia-free elderly subjects either referred to a memory clinic with minor complaints or healthy volunteers, and thus does not represent the general population. The AD and control group had ARWMC, a common finding in both populations, which may have influenced the size of the CC. However, there was no significant difference between the 2 groups in ARWMC score, and the scores were included as covariates in multivariable analysis. If a sample from a general elderly population would have been used, which presumably would have had a smaller incidence of ARWMC, it is possible that the relative difference in CC size would have been larger. Therefore, the reported difference in CC size in study I may be an understimation. On the other hand, since ARWMC score...
was not significantly different between the two groups, and since ARWMC is prevalent in AD patients, the control group may have yielded a more specific estimate of the CC atrophy which is due to AD specific pathology.

The strengths of study II and III include a very large sample size which has been well characterised clinically as well as imaging wise. Therefore, the study has a relatively high power and we were able to include relevant clinical and imaging biomarkers as covariates in the analysis. However, since subjects enrolled in the LADIS study all had ARWMC, the findings may not be applicable to a general population, but rather primarily apply to elderly populations with similar imaging-findings. Moreover, the longitudinal design of the study enabled the exploration of callosal tissue loss in subjects with ARWMC as well as exploring associations with cognitive and motor decline. Study II and III are, to our knowledge the first studies to do so. However, for study II it was a limitation that we were not able to examine how tissue loss was related to progression to specific subtypes of dementia diseases due to the relatively small number of subjects progressing. In study III, we were able to calculate composite score using test scores from several tests. This may be a more reliable method for quantifying the cognitive domains than the use of scores from a single test. However, a limitation of both the cognitive and motor tests are that they were not specifically designed to test functions which are thought to be reliant on the CC. The use of for example a test of bimanual abilities may have been more appropriate for establishing the role of the CC in motor functions in this age group. Indeed, the LADIS study, from which data for all three studies were used, was not specifically designed to study the CC. Due to the multicenter design of the LADIS different models of MR scanners were used with field strengths ranging from 0.5 – 1.5 T, which may have introduced confounding variance in measures of the CC. However, the use of phantoms helped to reduce inter scanner variability, and previous investigations have found that it is possible to combine morphometric data of the CC across different field strengths and scanner sequences [164]. Moreover, a flexible CC segmentation method was developed specifically for a multicenter setting, which has been applied several times in the LADIS study cohort [87,97,98,165]. Regarding the Witelson partitioning scheme, which was used for the present study, it should be considered whether this is the optimal solution given recent findings from DWI studies using tractography questioning the previously assumed somatotopy of the CC [166]. As previously stated, the Witelson partitioning scheme may not accurately correspond to the cortical areas from which the CC receives projections. However, the partitioning scheme is based on postmortem examination, and tractography is still a technique which needs validation from tracer studies. Moreover, tractography studies have also indicated that the interindividual variability is very high for the somatotropic organisation of the CC [54,166], and it has been suggested that this variability should lead to individual mapping of study participants CC prior to studying the CC [167].

We measured the area of the CC on the midsagittal slice. This measure has been used in numerous studies, and several postmortem examinations have found that this correlates with the number of axons in the CC [40,72,168]. However, future studies should consider using the parasagittal slices in conjunction with the midsagittal slice, which a recent study showed would reduce variability of area measures [169]. Moreover, although the CC is a 3-dimensional structure, it is to the best of our knowledge at present not possible to measure the volume of the CC due to its anatomy. When considering this, it is not unreasonable to assume that the area of the CC is the most optimal way of quantifying the CC.

CONCLUSION & PERSPECTIVES

As seen from the results presented in the thesis, the CC may play a role in dementia and age-related decline in cognitive and motor function. In patients with AD, we found atrophy of the posterior CC, and that the area correlated with cognitive decline over one year. We found that tissue-loss in this part of the CC also predicted progression to dementia over 3 years in subjects with ARWMC. Furthermore, tissue loss in these subjects was associated with processing speed, global cognitive function and motor function. However, the findings in the present studies should be regarded as exploratory, and the results need validation in further studies.

Regarding the role of the CC in dementia, the present study supports a model of CC atrophy in which changes in posterior CC are the first changes to be observed in the CC. However, based on findings from other studies, changes in the CC are most likely relatively late occurring events in the pathophysiological cascade in AD, compared to other structural changes such as hippocampal atrophy. Studies using DW-MRI may be able to detect microstructural changes in the CC, which precluded the macrostructural changes examined in these studies. We confirmed previous findings that CC atrophy is not associated with ARWMC in AD patients. Further imaging studies examining the association between CC atrophy, cortical atrophy and beta-amyloid are needed to further elucidate the underlying mechanisms behind CC atrophy.

We presented the first longitudinal studies examining the functional consequences of CC tissue loss measured over a period of 3 years in a large cohort of dementia-free elderly subjects with ARWMC. The findings establish that the CC plays a role in the age-related changes that occur regarding cognitive and motor function.

In conclusion, the findings underscore the relevance of the CC for different cognitive and motor functions in the aging brain and in dementia. Further studies are needed to confirm the findings presented herein, and new studies should aim at elucidating further the underlying mechanisms of changes in the CC. This includes the use of DW-MRI, as well as neuropsychological and motor tests specifically designed to assess functions related to the CC. Furthermore, the use of CC area measures as a biomarker in neurodegenerative dementias needs to be assessed.

SUMMARY

The overarching objective of the thesis was to investigate the morphological changes in the corpus callosum (CC) in aging and dementia in relation to its role in cognitive and motor decline. The CC is the largest white matter tract in the brain, containing upwards of 200 million axons, and is believed important for communication and interaction between the two cerebral hemispheres. Historically, the role of white matter, including the CC, in relation to cognitive function has often been eclipsed by the
Overall, the present findings confirm and extend the role of the motor function. Finally, CC tissue loss was also associated with impairment of executive functions, and that atrophy of the CC is associated with faster disease progression. This finding supports a model where posterior atrophy is the earliest changes in the CC in AD patients, with atrophy of anterior CC being a later pathological event. To further elucidate the role of CC atrophy in dementia, we examined a population of 329 elderly subjects, and found that a higher rate of tissue loss in posterior CC is associated with an increased risk of dementia. This study represents the first to examine CC in elderly subjects longitudinally. In the same cohort, we investigated whether impairment in specific cognitive domains were associated with CC tissue loss. Previous studies had shown that processing speed and executive functions may be particularly reliant on the CC. Our findings indicated that CC tissue loss leads to selective impairment of processing speed but not memory or executive function deficits. Finally, CC tissue loss was also associated with impairment of motor function.

Overall, the present findings confirm and extends the role of the CC in dementia and age-associated cognitive and motor deficits.

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many mild cognitive impairment measures and dementia severity and progression rates.


