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General Introduction
&
Aims and outline of the thesis
In this thesis the functional and anatomical connectivity of the brain at ‘rest’ will be discussed and explored. The initial motivation for performing research during a ‘resting-state’ is twofold: first, the fundamental interest in the ‘baseline state’ of the brain: broadening the knowledge of the brain’s intrinsic functional organization, and second the straightforward method of collecting images of brain activity at rest. The latter could be especially relevant in a medical setting as patients often have difficulties performing presented tasks. In the possible future scenario of using functional magnetic resonance imaging (fMRI) clinically for aiding the diagnosis of diseases such as Alzheimer’s disease (AD), resting-state fMRI has the advantage of being collected easily and quickly. Before the general aims and outline of this thesis are presented, three important topics in relation to this thesis will be briefly discussed.

The resting brain
The interest in the human mind and the function of our brain when ‘resting’, is certainly not novel. As illustrated by the citation of Seneca on page three of this thesis, this was already a topic of interest about 2000 years ago. A lot of knowledge about brain function has been gained in the last century starting with the development of the electroencephalogram by Hans Berger in the 1930’s making it possible to measure brain activity non-invasively, and the subsequent emergence of functional brain imaging techniques such as positron emission tomography (PET) in the 1980’s and fMRI in the 1990’s. One of the first studies into ‘resting state’ activity was performed by Andreasen and colleagues, by using PET they measured cerebral blood flow while subjects were engaged in ‘random episodic silent thinking’ (REST), i.e. when lying with eyes closed and thinking of whatever comes to mind (Andreasen et al. 1995). To date brain activity has mostly been measured during, at least, two conditions: a baseline condition when no external stimulus or just a fixation cross is presented and a task condition, measuring a specific cognitive function. To measure task-specific brain activity, activity during the baseline condition is subtracted from the task condition, using the baseline condition as a reference. In 1997, Shulman and colleagues (Shulman et al. 1997) noted that while applying this method in a meta-analysis of nine task-related PET studies, negative activation (i.e. deactivation) was observed in the same set of brain regions across these different studies (see figure 1). Initially little attention was focused on this observation until Raichle and colleagues published a paper a few years later in which they hypothesized that this set of brain regions constituted a ‘default mode’ of brain function, which is active in rest and deactivated when goal-directed attention is needed (Raichle et al. 2001). From then on the interest in the ‘resting-state’ of the brain has grown. The value of studying resting-state or intrinsic brain activity besides evoked activity is that additional brain processes can be studied. The idea that there is likely more to brain function than what is triggered by environmental stimuli is based on two bodies of information. First the energy burden of
intrinsic brain activity which far exceeds that of evoked activity. It has been estimated that 60% to 80% of the brain’s total energy budget is associated with brain activity while only 0.5% to 1% is associated with demands from the environment (Raichle, 2006). Brain networks thus appear to be predominantly intrinsically engaged and are occasionally perturbed by environmental inputs (Buzsaki, 2007). Second the striking organization of intrinsic activity as revealed by task-related activity decreases (Shulman et al. 1997) and the patterns of coherent blood oxygenation level dependent (BOLD) fluctuations measured at rest, the so-called ‘resting-state networks’ (RSNs) (Beckmann et al. 2005;Biswal, 1995). Currently many studies report both changes in activation and deactivation and more studies are being conducted without the presentation of external stimuli by measuring the brain ‘at rest’ (i.e. subjects are asked to lie down with their eyes closed and not to think of one thing in particular).

![Figure 1: Regions of the brain regularly observed to decrease their activity during attention demanding cognitive tasks (Shulman et al. 1997;Raichle et al. 2001)](image)

**Connectivity**

Classic approaches towards the relation between brain anatomy and cognitive function, based on the concept of functional segregation, may not be sufficient in understanding a complex dynamic system like the brain. As an alternative to study brain function of specific brain areas, studies of the connectivity of neural subsystems using several imaging techniques such as electroencephalography (EEG), magnetoencephalography (MEG) and magnetic resonance imaging (MRI), have attracted considerable interest in recent years (for a review see (Bassett and Bullmore 2006)). Inherent to resting-state FMRI is that the most common analysis approach, using a model of the BOLD response (e.g. modeling the alteration between task and control condition), is not applicable as data is acquired during only one condition (i.e. rest). Model-free network analyses are therefore a more suitable approach. The properties of brain networks and interactions among brain areas, however, have been
difficult to measure and to date still little is known about the intrinsic organization of the brain.

Connectivity between brain regions can be defined and studied both in terms of brain function (‘functional connectivity’) and anatomy (‘anatomical connectivity’).

**Functional connectivity**

Functional correlation and clustering methods based on signal fluctuations within brain systems provide a powerful means for examining network integrity. The basis of these techniques is that with functional measures such as EEG, MEG and FMRI, signal coherencies among brain areas can be detected. The notion of statistical associations between signals of brain activity as an indicator of functional interactions between brain regions is known as functional connectivity (Aertsen et al. 1989; Friston et al. 1993). In EEG and MEG interactions between brain areas can be determined by measuring the correspondence between signals derived from different channels, with each channel representing an electrode placed on the scalp over a predefined area of the brain. In FMRI most studies apply a region-of-interest cross-correlation analysis approach where the spatial pattern of signal fluctuations is estimated using a correlation analysis against a reference time-course derived from secondary recordings or the data itself (seed-voxel-based correlation analysis). More recently studies have applied model-free analyses such as an independent component analysis (ICA) (Beckmann and Smith 2004; Calhoun et al. 2001). ICA is a technique that separates linearly mixed signals in the data by maximizing their non-Gaussianity. This method, which is data-driven, is very suitable for exploratory studies as it is not biased towards specific brain regions, which is the case in the previously mentioned region-of-interest cross-correlation analysis.

**Anatomical connectivity**

While knowledge of the functional connectivity of the human brain has improved in recent years less is currently known about the underlying anatomical connectivity. The main cause is lack of techniques and technical limitations of existing techniques to measure anatomical brain connectivity in vivo. Anatomical connectivity is most effectively studied by injecting tracers, i.e. chemical substances which are taken up by the cell body and then transported to the axon terminal or vice versa. The nature of this method excludes human subjects because the visualization of the connectivity patterns is performed in vitro (i.e. the subject has to be sacrificed), consequently, animal models have been used to study anatomical connections in the brain. Recently a technique has been developed that allows us to study anatomical connectivity in vivo: diffusion tensor imaging (DTI). DTI is based on the measurement of characteristics of water diffusion, i.e. fractional anisotropy (FA), apparent diffusion coefficient and diffusion...
direction (principle eigenvector), in the brain. The fact that water molecules will more readily diffuse along the major axis of the fiber bundle than perpendicular to it (Moseley et al. 1990) provides the basis for this in vivo visualization of white matter tracts (Le Bihan, 2003). One of the most important factors affecting the FA value is the integrity of axons and their myelin sheaths, the measurement of FA values can therefore be used for the detection of degeneration of neural tracts (Gupta et al. 2006).

Aging and Alzheimer’s disease as disconnection syndromes
Normal aging is related to cognitive decline even in the absence of disease. Attention, information processing and working memory are particularly compromised in the elderly (Craik and Salthouse 2000; Salthouse and Ferrer-Caja 2003). In AD a more prominent decline in cognitive function is observed. Memory function is primarily compromised along with impairment in at least one other cognitive domain such as language or visuospatial abilities (Lindeboom and Weinstein 2004; Pasquier, 1999). In addition to cognitive impairment, aging and AD are also associated with changes in brain function and anatomy. It has been suggested, based on evidence mainly from neuropathological, electrophysiological and neuroimaging studies, that aging and AD are disconnection syndromes (Delbeuck et al. 2003; O’Sullivan et al. 2001). This disconnection hypothesis posits that decline in normal aging and AD emerges from changes in connections between brain areas, in addition to dysfunction of specific areas. In this thesis connectivity changes in normal aging and AD will be investigated.

Aims and outline of the thesis
The general aims of this thesis are to study the organization of intrinsic functional brain connectivity using FMRI and to explore connectivity changes in aging and AD. Additional, strongly related topics presented in this thesis cover: 1) the investigation into the difference in sensitivity of model-free measures and task-related analysis approaches in detecting connectivity changes in AD, 2) the assessment of anatomical connectivity in aging and AD by measuring white matter integrity, and 3) the effect of pharmacological intervention on intrinsic functional brain connectivity.

The outline of this thesis is as follows: The next chapter, chapter 2, introduces the application of a novel technique to perform a model-free group analysis to investigate functional connectivity of resting-state FMRI data of healthy young subjects, and subsequently explores the consistency of the resulting ‘connectivity maps’ or RSNs. In chapter 3 connectivity changes in aging and AD are studied. Chapters 3.1 and 3.2 present our research into the effects of normal aging and AD, respectively, on the activity of RSNs. The study presented in chapter 3.3 investigates whether a connectivity measure like the one applied in the previous chapters, is more sensitive than model-based methods to detect task-related FMRI
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activation changes in AD. Given that disruption in functional connectivity is thought to be related to impairment in anatomical connectivity (i.e. white matter integrity), we additionally used DTI to study the differences in anatomical connectivity between healthy older subjects and patients with mild cognitive impairment (MCI) and AD (see chapter 3.4). To understand more about intrinsic brain activity we additionally performed a study, presented in chapter 4, into the effects of pharmacological manipulation on intrinsic brain activity by administrating the stress hormone hydrocortisone to young healthy male subjects. Finally, in chapter 5 a summary of the previous chapters is provided along with a general discussion and suggestions for future directions.
References


