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INTRINSIC BRAIN ACTIVITY IN HEALTH AND DISEASE

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INTRINSIC BRAIN ACTIVITY IN HEALTH AND DISEASE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Dedicated to the rested brain's intrinsic motivation to explore, expand and contribute

ABSTRACT

The main part of the brain's energy need is to support housekeeping functions and internal information processing, regardless of external tasks. Cognitive brain imaging, aimed at relating mental phenomena to neurophysiological processes, has conventionally investigated the brain activity induced by external stimuli. In contrast, resting state functional Magnetic Resonance Imaging (rs-fMRI) aims to characterize the spatiotemporal properties of the ongoing baseline brain activity.

In the projects constituting the current thesis, we have used rs-fMRI to investigate effects of neuropharamcological administrations, long-term physical exercise and to characterize central pain processing in rheumatic pain conditions. **Study I** is a randomized, cross sectional placebo study, in which healthy subjects were administered Parkinson medications (L-dopa), anxiolytics (oxazepam), or placebo. Our a priori hypothesis of preferential modulations of connectivity of brain regions with high density of target receptors was not confirmed. Instead, oxazepam was associated with increased connectivity of cardinal hubs within the default mode network, and interestingly, a decoupling of the amygdala. L-dopa, on the other hand, primarily decreased connectivity, particularly between amygdala and bilateral prefrontal gyri.

In **studies II-IV** we investigated rheumatic pain patients. In **study II** we compared a fibromyalgia (FM) cohort and healthy controls (HC) with regard to functional brain connectivity of particularly cerebral pain regions. Conducting both data driven independent component analysis (ICA) and seed correlation analysis (SCA), we observed a weaker coupling between pain regions and sensorimotor brain areas in the FM group. Across groups, pain sensitivity correlated with e.g. increased connectivity between insula and the posterior cingulate cortex.

Physical exercise is a potent reliever of FM symptoms. In **study III**, we investigated the effects a three months physical training intervention for FM patients. Following exercise, patients reported decreased symptom gravity, and the FM associated hyper-connectivity identified at baseline was partly normalized.

In **study IV**, we investigated the extent to which exposure to chronic pain for patients with rheumatoid arthritis (RA) was reflected in functional connectivity of pain regions. Overall, RA patients had elevated connectivity, particularly between frontal midline areas and bilateral sensorimotor cortex.

Taken together, we have shown that short-term neuropharmachological interventions, a three months physical exercise intervention as well as long-term rheumatic pain exposure, all are accompanied by changes in intrinsic brain activity. Although the functional significance of the observed group differences in connectivity warrants further investigations, the evidences presented here support the notion that rs-fMRI could prove useful for diagnosing neuropsychiatric conditions and evaluating interventions in the future.

SAMMANFATTNING

Merparten av hjärnans energikonsumtion används till bearbetning av endogen information och upprätthållande av hjärnans homeostas. Funktionell magnetresonansavbildning (fMRI) är en av de främsta metoderna för att lokalisera mentala processer i hjärnan. Detta uppnås genom att jämföra regionala förändringar i hjärnaktivitet orsakad av yttre stimuli, med hjärnaktivitet under vila eller annan kontrollbetingelse. En nyare, kompletterande användning av fMRI är att studera hjärnaktivitet i frånvaro av externa stimuli eller uppgiftsinstruktioner. Denna metod kallas fMRI av spontan hjärnaktivitet (eng. ”resting state fMRI”).

I denna avhandling presenteras fyra olika studier i vilka fMRI har använts för att mäta spontan hjärnaktivitet. I **studie I** undersöktes effekterna av två typer av frekvent ordinerade psykoaktiva substanser: L-dopa (mot Parkinson) och oxazepam (mot oro, ångest och sömnsvårigheter). Jämfört med en placebogrupp uppvisade oxazepam-gruppen starkare funktionell konnektivitet (definierade som korrelation mellan tidsserier) inom det så kallade standardnätverket (DMN), och svagare konnektivitet mellan t.ex. amygdala och temporala cortex. L-dopa associerades framförallt med starkare konnektivitet, t.ex. mellan amygdala och frontala regioner.

I **studie II** karaktäriserade vi spontan hjärnaktivitet hos fibromyalgi (FM) patienter. Patienter jämfördes med friska kontrollpersoner i en oberoende komponentanalys samt med avseende på konnektivitet för 159 regioner placerade i cerebrala smärtområden. FM associerades i huvudsak med försvagad konnektivitet, t.ex. mellan smärtområden och sensorimotor cortex. Vidare var smärtkänslighet i båda grupperna korrelerad med ökad konnektivitet mellan t.ex. insula och posteriora cingulära cortex.

I **studie III** undersökte vi effekterna av en tre månaders sjukgymnastikbehandling för FM patienter. FM-symptom minskades efter träningsinterventionen. Samtidigt återställdes vissa av de funktionella kopplingar som i studie II identifierats som avvikande hos patienterna. I synnerhet förstärktes konnektiviteten mellan insula och sensomotoriska områden.

I den sista **studien** (**IV**) undersökte vi hur långvarig kronisk smärta bland patienter med reumatoid artrit avspeglades i konnektivitetsmönstret för samma 159 smärtregioner som undersöktes i studie II. Vi detekterade en generellt förhöjd konnektivitet, primärt mellan frontala kontrollregioner och bilaterala sensomotoriska områden.

Sammanfattningsvis har vi påvisat att spontan hjärnaktivitet kan moduleras av i) kortvarig medicinsk behandling, ii) tre månaders träningsintervention, samt iii) långvariga reumatiska smärttillstånd. Även om det återstår att i oberoende studier med hög statistisk tillförlitlighet (s.k. “power”) utvärdera den kognitiva, biologiska och kliniska innebörden av dessa förändrade konnektivitetssmönster, så belyser studierna den potentiella användningen av fMRI av spontan hjärnaktivitet för neuropsykologisk diagnostisering och utvärdering av behandlingar.

LIST OF PUBLICATIONS

I. **Flodin P**, Gospic K, Petrovic P, Fransson P. (2012). Effects of L-dopa and oxazepam on resting-state functional magnetic resonance imaging connectivity: a randomized, cross-sectional placebo study. *Brain Connectivity*, 2:246-53.

II. **Flodin P**, Martinsen S, Löfgren M, Bileviciute-Ljungar I, Kosek E, Fransson P. (2014). Fibromyalgia is associated with decreased connectivity between pain- and sensorimotor brain areas. *Brain Connectivity*. 4:587-94.

III. **Flodin P**, Martinsen S, Mannerkorpi K, Löfgren M, Bileviciute-Ljungar I, Kosek E, Fransson P. (*2015*). Normalization of aberrant resting state functional connectivity in fibromyalgia patients following a three months physical exercise therapy. *NeuroImage:Clinical. (in press)*

IV. **Flodin P**, Martinsen S, Altawil R, Waldheim E, Lampa J, Kosek E, Fransson P. (*Submitted*). Intrinsic brain connectivity in chronic pain: A resting- state fMRI study in patients with rheumatoid arthritis.

LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

I. Fransson P, **Flodin P**, Seimyr GÖ, Pansell T. (2014). Slow fluctuations in eye position and resting-state functional magnetic resonance imaging brain activity during visual fixation. *European Journal of Neuroscience.* 40:3828-35.

II. Martinsen S, **Flodin P**, Berrebi J, Löfgren M, Bileviciute-Ljungar I, Ingvar M, Fransson P, Kosek E. (2014). Fibromyalgia patients had normal distraction related pain inhibition but cognitive impairment reflected in caudate nucleus and hippocampus during the Stroop Color Word Test. *PLoS One*. 9(9)

III. Martinsen S, **Flodin P**, Berrebi J, Löfgren M, Bileviciute-Ljungar I, Mannerkorpi K, Ingvar M, Fransson P, Kosek E. (*In manuscript*). The role of long-term physical exercise on performance and cortical activation during the stroop color word task in fibromylagia patients.

CONTENTS

LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
ACR	American college of rheumatology
BOLD	Blood oxygen level dependent
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CMRO2	Cerebral metabolic rate of oxygen
CompCor	A principal component based method for reduction of noise
COV	Covariance
CSF	Cerebral spinal fluid
DAS28	Disease activity score 28
DMN	Default mode network
DSI	Diffusion spectrum imaging
DTI	Diffusion tensor imaging
ECoG	Electrocorticography
EEG	Electroencephalogram
EPI	Echo planar imaging
fALFF	Fractional amplitude of low frequency fluctuations
FD	Frame wise displacement
FID	Free induction decay
FIQ	Fibromyalgia impact questionnaire
FM	Fibromyalgia
fMRI	Functional magnetic resonance imaging
FSL	FMRIB software library
GABAa	Gamma aminobutyric acid a
GLM	General linear model
GSR	Global signal regression
HC	Healthy control

IC	Independent component
ICA	Independent component analysis
ICC	Intraclass correlation coefficient
LFP	Local field potential
MEG	Magnetoencephalography
MELODIC	Multivariate exploratory linear optimized decomposition into IC
MNI	Montreal neurological institute
MR	Magnetic resonance
MUA	Multi unit activity
NSAID	Non steroidal anti-inflammatory drugs
P50	a pain pressure measure
PCA	Principal component analysis
PCC	Posterior cingulate gyrus
PET	Positron emission tomography
RA	Rheumatism arthritis
ReHo	Regional homogeniety
RF	Radio frequency
rs-fMRI	Resting state fMRI
RSN	Resting state networks
TR	Repetition time
SCA	Seed correlation analysis
SF36BP	Short form 36 body pain
SPM	Statistical parametric mapping
TE	Echo time
TMS	Transcranial magnetic stimulation
VAS	Visual analog scale
WM	White matter

1 INTRODUCTION

Mental and neurological disorders pose a large and growing challenge for the health system world wide (Whiteford et al., 2013), and top the disease burden (years lived with disability) in Sweden (Allebeck et al., 2006). The need for an increased and deeper scientific understanding of the neural processes that subserve mental processes is widely acknowledged, which also is reflected in the many large-scale brain research programs that recently have been launched (Reardon, 2014). The intimate and fundamental role that the central nervous system holds in human life makes neuroscientific research extraordinarily worthwhile, with implications not only for health care, but likely also for the educational and the juridical systems. Ultimately, the age-old philosophical quest regarding the relationship between conscious experience and the physical realm is finally systematically addressed using the scientific method.

Like most branches of science, neuroscience has developed rapidly during the last century. Among the subfield of neuroscience, the discipline of cognitive neuroscience has undergone an exponential increase in number of publications. The now dominating brain imaging technology for investigating neural correlates of psychological phenomena is functional magnetic resonance imaging (fMRI). Since the first human fMRI study by Belliveau et al. (1991), more than ten thousand fMRI studies has been published (figure 1).

A common denominator for all studies contained in the current thesis is the methodology of resting state fMRI (rs-fMRI). Although resting state brain imaging here has been applied to different research topics (such as neuropharmacological manipulations, rheumatic pain and physical exercise treatment), the emphasis will in the following be on the biological and functional significance of resting state connectivity. Since the analytic arsenal and the scope of the rs-fMRI field is huge and continuously expanding, I will limit the discussion to methodological issues and resting state measures that are most relevant to the current research projects. The interpretations of rs-fMRI, including the reported results in the latter part of this thesis, critically rely on the meaning of the rs-fMRI signal. Like all methodologies, rs-fMRI suffers limitations that need to be addressed in order to maximize its use and future development. Hence a substantial part of the following pages will be devoted to this.

1.1 BRIDGING MIND AND MATTER – WHAT ROLE CAN BRAIN IMAGING PLAY?

Science deals with interpersonally shareable symbols such as quantities. A core feature of phenomenal consciousness (or the ability to experience) is the first person access and subjectivity. Subjective experience (qualia) does not easily lend itself to quantification. Any attempts to measure the “raw feel” in a given situation inevitably rely on arbitrary decisions and approximations to a high degree, in contrast to quantifications in the natural sciences. This poses great challenges for the science of mind. In the contemporary philosophy of mind, “the hard problem of consciousness” was originally coined by (Chalmers, 1995), and refers to the issue why and how physical processes give rise to subjective experience at all. The “easy

problems", on the other hand, pertained to how (eventually overt) behavior (including information processing) comes about. For practical reasons, the main focus in cognitive neuroscience is to investigate the latter ones.

A goal of cognitive neuroscience is to describe the necessary and sufficient neurophysiological processes subserving particular cognitive processes (here defined as all mental abilities related to knowledge and information processing, including cerebral sensorimotor processes). Although brain imaging is a mainstay in cognitive neuroscience, it generally falls short when used to establish either the necessary or the sufficient neurophysiological conditions for mental operations. To determine whether a putative causal factor (such as a neuronal process) is necessary for a particular phenomenon (e.g. a cognitive process), the factor in question must be isolated and independently perturbed, to detect if its absence abolishes the effect. Brain imaging merely passively record and never modulate brain activity. Accordingly, the necessity of a particular brain state to bring about cognition cannot be determined on the basis of imaging alone. Thus, although brain imaging can be successfully used to identify neuronal correlates of cognitive phenomena, it fails to selectively manipulate neuronal activity. To accomplish this, complementing methodologies such as animal lesioning or transcranial magnetic stimulation (TMS) are required. Theoretically, brain imaging could identify the brain regions that are sufficient for a given cognitive task. In practice, the normally relatively low statistical power in imaging studies likely prevents this. Besides, the brain imaging technologies at hand might not by themselves pick up all the relevant brain activity (e.g. EEG is insensitive to glia cell activity, and the sluggishness of the hemodynamic response likely makes fMRI blind to critical properties of neuronal firing rates etc.). Furthermore, brain activity that is essential and associated with a certain cognitive function could be shared with the control conditions (e.g. brain activity supporting life upholding functions, arousal etc.), why such activity would typically be controlled for (cf. subtraction methodology, section 1.1.1.1). Therefore it is improbable that brain imaging could delineate the sufficient brain processes subserving a certain cognitive process.

Clearly, brain imaging is not capable of drawing strong conclusions regarding the causal relationships between brain activity and cognition. Still, brain imaging can, and has played, an essential role for understanding the relationship between mental and biological processes. Firstly, brain imaging could provide guidance for studies that in principle could detect causal relationships. Secondly, brain imaging could inform cognitive science without references to causal relationships, e.g. through forward and reverse inference (see section 1.1.3 below).

1.1.1 Task vs. rest

1.1.1.1 Pure insertion

Brain imaging in cognitive neuroscience has conventionally employed task-based paradigms. The fundamental logic behind task related study designs relies on the cognitive subtraction methodology. Cognitive subtraction is (in most cases) implicitly based on the assumption of pure insertion (Posner, 1978), also known as the 'differential principle'. This principle was formulated by the physiology researcher Donders, active in the field of mental chronometry in the 19th century. The subtraction methodology aims to identify the behavioral and neurophysiological processes that are involved in a certain task condition of interest, condition "A", by comparing it with a baseline or control condition "B". The two conditions are compered by simply subtracting the neurophysiological data that was recorded during the condition B from the data of condition A. By applying this fundamental logic (including its refined elaborations, such as of factorial designs that allow for investigations of interactions) to a wide variety of research questions, one has obtained the majority of the cognitive neuroscientific findings that charts the functional brain anatomy. Despite limitations of the underlying assumption of pure insertions (such as non-linear effects due to learning, neuronal adaptations and non-linear interactions between experimental factors, as well as unavoidable imperfections of the control conditions) (Friston et al., 1996), the subtraction methodology has been highly successful, and continues to play an important role in experimental designs in cognitive neuroscience. To date, most of the software used for performing statistical analysis on fMRI data rely on the implementation of a massive univariate statistical testing and is based on the general linear model that naturally lends itself to comparing experimental conditions by subtracting them with each other. Likewise, the common way of presenting the results of fMRI studies is in the form of activation maps where areas that are significantly more activated for one condition relative another are color-coded "blobs".

1.1.1.2 The birth of resting state imaging

However, the subtraction methodology has not had monopoly as a guiding principle on how to conduct cognitive neuroscientific research. When Hans Berger introduced the electro encephalogram (EEG) in the late 1920s, he recorded the spontaneous background activity from neurons in humans, and determined the spectral characteristic of epochs without reference to any control conditions. Thus, since its very origin, electrophysiological investigations has relied on measurements of the baseline brain activity, and resting state brain activity continues to be a major explanandum both in research using magneto encephalography (MEG) and EEG, as well as intracranial recordings. The comparably high temporal resolution of electrophysiological measures allowed for interesting measures of the baseline brain activity, primarily by frequency decompositions using Fourier transformation. For brain recoding technologies based on hemodynamics (like fMRI), the brain's baseline activity was until recently a rather ignored field. Although the baseline metabolism had been investigated using fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) since the early 1980s, studying baseline activity with the less invasive and increasingly

popular fMRI technique would not have rendered meaningful results. The reason for this is that the absolute magnitude of the blood oxygen level depended (BOLD) signal, which is the predominant contrast mechanism used for tracking neuronal hemodynamics in fMRI, lacks intrinsic interpretation in contrast to the signal amplitude of e.g. FDG-PET. Instead, the meaning of the fMRI signal is conventionally obtained by contrasting two conditions employing the subtraction methodology as described above (section 1.1.1.1). However, in a seminal paper from 1995, Biswal and colleagues employed a new analytic approach of fMRI data where they investigated the spontaneous fluctuations of the BOLD signal when subjects were just resting. They reported how the resting state BOLD signal in cortical motor regions oscillate primarily at low frequencies (<0.1 Hz), and that these oscillations were bilaterally synchronized (Biswal et al., 1995). Biswal noted that the BOLD signal during "baseline" or rest periods displayed a functionally relevant spatiotemporal organization, and this observation became the starting point for an entirely new subfield of the fMRI research; the field of rs-fMRI.

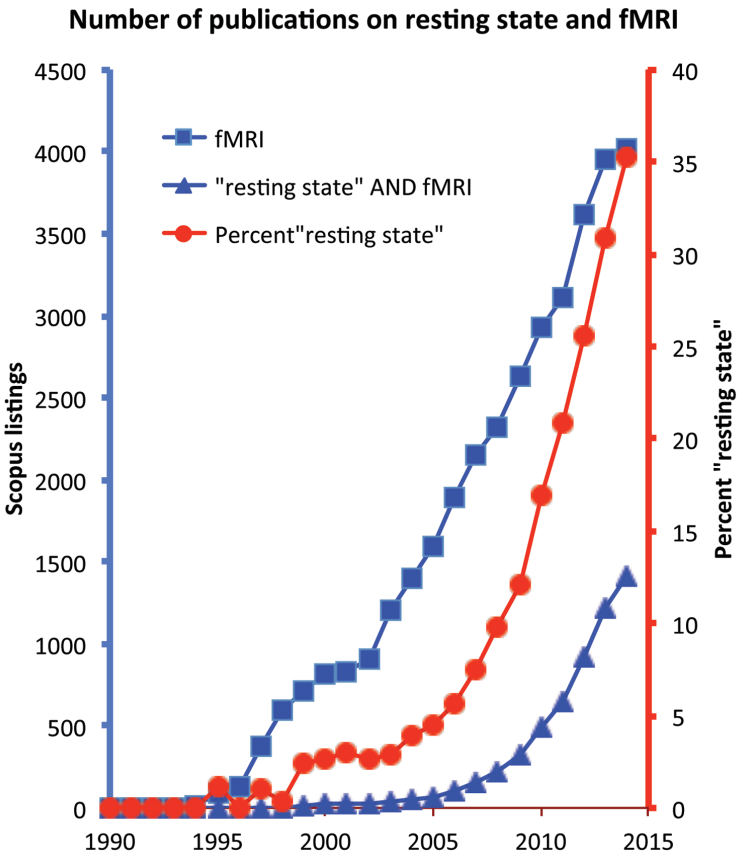

Figure 1. The increasing share of resting state studies among fMRI studies. The graph show number of hits on Scopus retrieved from the searches "(TITLE-ABS-KEY (fmri)) " and "(TITLE-ABS-KEY (fmri) AND ("resting state")) " on publications between 1990 and 2014. Red graph illustrates the percentage of hits using the latter search criteria relative to the number of hits obtained by the former. The search was conducted 24th June 2015.

In parallel, Marcus Raichle, an early adopter of the subtraction methodology for localizing mental operations (Posner et al., 1988), published a paper that should become another cornerstone in cognitive neuroscience (Raichle et al., 2001). Their approach was to reverse the contrast of interest of task based PET studies covering a wide variety of task conditions (including attention, working and long-term memory, and language tasks). The authors observed a strikingly similar set of brain regions which was consistently more activated during rest compared to cognitive task in the diverse experimental paradigms that were reviewed. These regions covered several medial brain areas, including medial ventral prefrontal cortex and posterior cingulate cortex (PCC), as well as bilateral inferior parietal cortex. The baseline or rest conditions that previously had been overlooked and subtracted from the task evoked brain activity of interests, was now shown to possess

spatial regularities and characteristics that were intriguingly universal. This laid the foundation of the so-called default mode network (DMN).

1.1.2 The network view of the brain

By adopting the procedures set out by the preceding discipline of cognitive science and even phrenology, cognitive neuroscience relied on task based fMRI designs that was largely confined to the methodology of dividing cognition into distinct mental operations, that in turn were localized to certain brain regions.

This approach has rendered an impressive amount of data about the functional anatomy of the brain, where virtually every cm^3 of the grey matter in the human cerebrum has been associated with at least one function by any of the tens of thousands cognitive brain imaging studies that has been published. Although certain cognitive processes are known to predominantly rely on a particular brain region -a typical example is face recognition that are localized to the fusiform gyrus-, there are increasing data that supports the idea that the fundamental organizational principle of the brain is distributed processing. In other words, the neural representation of high-level information does not generally consist of a single brain area or a neuron (e.g. the so called "grand mother cells"). Rather, neuronal representation of cognitive processes are likely distributed, sparse, and subserved by functionally integrated brain regions that are separate in space, -networks that operate on different spatial and temporal scales (Sporns, 2015; Fox et al., 2012). These networks can in turn connect and disconnect with other networks and subcomponents. The emerging network view of the brain thus dismiss the simplified picture of the brain as constituted by a mosaic of non-overlapping, independent areas that are uniquely devoted to a specific set of high level mental operations. A description of such multistage organization and dynamics easily become more complex than the typical descriptions that read along the lines of "one structure – one function", since the network view attempt to take into account the different combinations of connections between brain areas (or nodes), as well as the dynamics of the network interactions. However, the interpretations of the functional significance of networks are still largely derived from previous studies that localize function to particular brain areas. The development of meta-analytic tools (e.g. neurosynth.org) and the accumulation of further findings of classical mappings of function to brain structure will increase their generalizability and use. Rs-fMRI has played a central role for characterizing the brain's network organization. A reason for this is that rs-fMRI naturally allows for investigation of simultaneous brain activity across distributed brain regions, in contrast to task-fMRI that conventionally has focused on localized, task-induced brain activations.

1.1.3 Forward and reverse inference

The success and popularity of functional brain imaging (in which rs-fMRI has an increasing importance), is due to its potential in helping us understand the neurophysiological processes that underlies different aspects of cognition. As previously mentioned, the dominating approach has been to identify mental operations of interest (such as encoding of semantic

memory), then formulate a control condition that contains all confounding processes (e.g. the visual properties of the task stimuli), and subtract brain activity of the latter condition from the recordings of the former condition. Ideally, cognitive brain imaging thus creates associations between a certain mental operation A to a certain brain response X. This kind of function-to-structure mapping constitutes the result section of virtually all cognitive brain imaging articles. Although it is fascinating in itself, narrowing down the neural substrate of a cognitive process A, from "somewhere in the brain" to a specific location (or, -perhaps more apt- a certain spatiotemporal activation pattern) is arguable of little use for understanding cognition itself (Coltheart, 2006). Still, the more elaborated and mechanistic models of cognition and its neural underpinnings commonly rely on initial work of that kind. Cognitive processes can be inferred from brain imaging through two kinds of approaches. One is forward inference, which is based on the hypothetico-deductive method. It corresponds to the dissociation logic that since long has been employed in neuropsychological research. Forward inference requires a priori formulation of two (or more) competitive psychological theories that can be supported or refuted based on recordings of neurophysiology. That is, the only assumption of forward inference is that a certain cognitive process is not instantiated by different brain regions during different conditions within the same experiment. A common example of how brain-imaging data is used to inform cognitive science through forward inference regards recognition memory (Henson, 2006): Two competitive views on recognition memory had been proposed, namely the single process theory vs. the dual process theory. The first holds that familiarity (or knowing) is just a weak form of recollection (or remembering), but qualitatively the same (in terms of cognitive ontology) although on opposite sides of the continuum ranging from weak to strong memories. The dual process theory claims that familiarity and recollection are two distinct processes. The fMRI observation of largely non-overlapping neural correlate of familiarity and recollection have been taken as a support for the dual process theory (Henson, 2006).

Forward inference is limited not only by the requirement of a priory formulated competitive cognitive theories, whose quality poses an ultimate limitation of the interpretations that can be drawn from the experimental results. Another major challenge is how to determine what constitute qualitatively different brain activations, which still is under debate although preliminary criteria have been formulated (Henson, 2006). Finally, to the extent one fails to obtain high statistical power, an absence of brain response in one condition over another could also be a false negative finding (i.e. a type two error), thus preventing inference based on dissociation logic.

A second approach by which brain-imaging data can inform cognitive theories is through reverse inference (Poldrack, 2011). It denotes inferences on the sort of cognitive processes that occur, based on observed brain activations. For reverse inference to be logically valid, it is required that the brain activation in question is uniquely associated (by earlier studies) with a certain cognitive process. However, this is seldom the case. Rather, it is likely that any given brain area detectable by fMRI has more than one function. For instance, areas such as the insula or anterior cingulate cortex are frequently activated throughout a wide range of

cognitive tasks. Yarkoni et al. (2011) estimated that activity in anterior cingulate cortex is reported in 20% of all functional brain activity studies, making any interpretations of the functional role of brain activations in such an area very weak. Thus, in order to increase the validity of reverse inference, more selective and robust associations between cognitive processes and brain activations are needed. The quality of the functional anatomy in turn, is fundamentally limited by the validity and ontological status of the cognitive constructs, as well as the anatomical labels on which cognitive constructs are mapped (Brett et al., 2002). As a good first step, collaborative projects aimed to systematically formulate a comprehensive cognitive taxonomy based on a large body of cognitive neuroscience literature, have been initiated (cognitiveatlas.org and brainmap.org). A second way to strengthen reverse inference is to increase the prior probability of the cognitive process in question. This can be accomplished by designing the experiment in such a way that the number of possible cognitive functions that is compatible with the associated observed brain activity is decreased, (Poldrack, 2006).

Reversed inference, due to its probabilistic rather than logically necessary conclusions, is a form of abductive reasoning (i.e. reasoning to the best explanation) that could be used for stipulating new hypothesis to be investigated further.

1.2 RS-FMRI MEASURES

The arsenal of analytic tools to characterize rs-fMRI data has undergone a rapid development and expansion the last decade. Despite a rich variety of analytical rs-fMRI measures, they share a common basis. All analytic approaches employed in the current study make use of the fact that the rs-fMRI BOLD signals oscillate. The amplitude of these oscillations is typically less than 5% of the average signal intensity of a given time series (which is in the same order of magnitude as the signal changes observed in task paradigms). The properties of these intrinsic oscillations are not fully understood, although there are accumulating evidence that they have a functional (i.e. behavioral or cognitive) relevance to a certain degree (see section 1.5.2). The power distribution of the intrinsic oscillations is skewed towards the lower frequencies (in accordance with the 1/f power law), and this is one of the main reasons why the frequency window of interest commonly is chosen between 0.01-0.1 Hz. In the studies that are describe here, we have used both resting state measures reflecting the degree of BOLD-signals variation across brain regions, as well as a measure that characterizes the frequency distribution of BOLD-signal oscillations.

1.2.1 Measures based on covarying BOLD signals

The by far most common principle underlying rs-fMRI analysis is the mapping of the spatiotemporal patterns of covarying fMRI time series. Functional connectivity is defined as the temporal correlation of BOLD signals, and the full brain correlation maps are called connectivity maps or resting state networks, where resting state networks denotes connectivity maps that are frequently reported and reproduced (see Damoiseaux et al., 2006).

1.2.1.1 Seed correlation analysis

The principle behind seed correlation analysis (SCA) is intuitive and straightforward. SCA was the original analytical method used for probing rs-fMRI (Biswal et al., 1995), and is still one of the most common methods for investigating covariation of BOLD-signals across brain regions. Following preprocessing of functional data (typically involving several additional steps compared to conventional task evoked brain imaging, see the method sections), a seed region is defined and the BOLD signals of its constituting voxels are extracted and averaged. The time series is subsequently related to the times series of the remaining voxels of the brain image (or alternatively, to a set of other pre-defined regions of interest –ROIs–, in the case of ROI-to-ROI analysis), either by conducting linear regression or Pearson correlation. Thus one generates a functional connectivity map depicting voxels or regions of the brain whose BOLD signals covary with the BOLD signal of the seed region (see Figure 2).

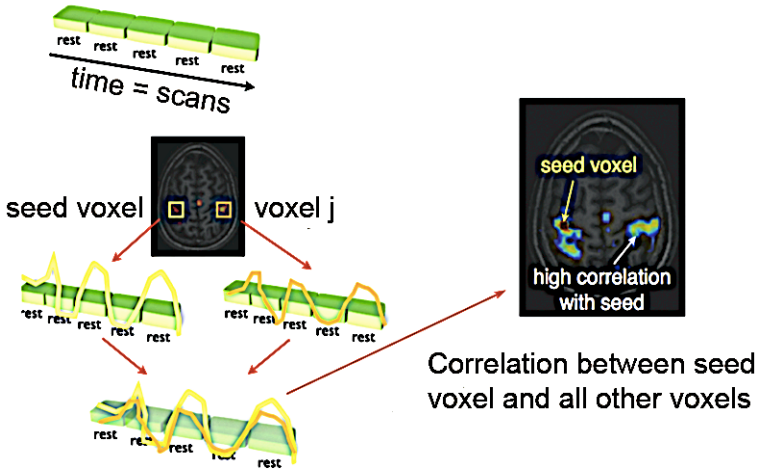

Figure 2. The principle behind seed correlation analysis (SCA). Seed regions are defined, and the pertaining time series is related to the time series of the remaining brain, rendering whole brain connectivity maps.

The relationships between time series are quantified using massive univariate approaches of linear regression or correlation analysis. Bivariate regression and bivariate Pearson correlation differs in how the covariance between the two signals are normalized. In bivariate regression, the covariance (COV) is normalized by the variance of the independent variable (σ_I^2), rendereing a measure (beta estimate, β) that reflects how many units the dependent variable (D) changes for each unit change of the independent variable (I) (i.e. here the time series of the seed region).

$$\beta = \frac{COV(D,I)}{\sigma_I^2}$$

Pearson correlation coefficient, on the other hand, normalizes the covariance by the standard deviation of the first variable (σ_I) times the standard deviation of the second variable (σ_D).

$$r = \frac{COV(D,I)}{\sigma_D \cdot \sigma_I}$$

Thus, the Pearson coefficient is a unitless measure of the degree to which two variables are linearly related, and its sign denotes the directionality of this relationship. The two can be converted to each other, and regression or Pearson correlation normally gives similar results. Since the correlation coefficient is not normally distributed (in particularly for strong positive

or negative correlation values in a population), the correlation coefficient needs to be transformed (z') using fisher z-transformation according to

$$Z' = 0.5 \cdot ln\left(\frac{1+r}{1-r}\right)$$

before further (e.g. group) analysis based on the general linear model (GLM) is conducted, due to the normality assumption of the GLM.

One critical aspects of SCA is the choice of seed region. The location and size of the seed highly influence the resulting connectivity map in ways that are not always desired, inducing variance between studies and weaker overlap of connectivity maps across studies (Cole et al., 2010). On the other hand, a benefit of the a priori definition of seed regions is that it allows for investigation of the functional connectivity of very localized parts of the brain. Seeds are commonly defined based on the MNI (Montreal Neurological Institute) coordinates of peak activations that have been reported in the previous literature, or those that are revealed through meta-studies. Less commonly, seed regions can be defined on the basis of anatomical labels or landmarks, such as gyri or sulci. Alternatively, seeds could be defined based on functional localizers obtained from preceding task-evoked fMRI sessions that yield either subject specific regions of interest, or regions revealed from group analyses. Since the functional anatomy varies across subjects, subject specific functional localizers provide powerful means to target the relevant connectivity, assumed that relevant task based fMRI data is available. However, functional connectivity maps are conventionally summarized on a group level, which requires prior normalization. Thus, the intra-individual specificity gained by using subject specific seed regions (and possibly conducting SCA in native space), is partly lost when the subject specific connectivity maps are normalized. Subject specific seed regions can also make it difficult to relate the findings to previous studies e.g. through meta-analyses.

In the studies that form this thesis, we performed SCA using seeds defined on the basis of either meta-analytic results (**study II-IV**), or based on anatomical regions as reported in the literature (**study I**).

1.2.1.2 Independent Component Analysis

Independent Component Analysis (ICA) is a data driven approach for decomposition of a multivariate signal into additive subcomponents. The currently dominating application of ICA resting state data analysis is to identify maximally statistically independent spatial maps. These independent components (ICs), or spatial maps, highlight voxels that shares similar (or correlated) time courses. Although both the number of spatial independent components (ICs) and the choice of ICs on which to perform inferential group statistics are determined by the user, the spatial maps themselves are generated in a data driven fashion. Thus, for many purposes, ICA is advantageous to SCA in that ICA does not introduce seed selection bias. On the other hand, even though ICA frequently reveals neuronal networks that can be confirmed using SCA, ICA also commonly yield partial network coverage, or networks that are a

superposition of (expected) networks, which sometimes makes it difficult to relate ICA results to the previous literature. Similarly, there is no consensual way of how to compare spatial ICs between individuals in order to perform group analysis. However, two approaches have become dominating. One is dual regression, as implemented in the FSL software MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) (Beckmann et al., 2004) (see Figure 3). In dual regression, the rs-fMRI data is concatenated over subjects (using multi-session temporal concatenation in MELODIC). Spatial ICA is performed on the concatenated data. The retrieved group-averaged spatial maps are used as spatial regressors in the subjects' 4D resting state data. This yields subject specific time series that correspond to each of the group ICs. Finally, these time series are used as temporal regressors in a conventional linear regression. This results in subject specific versions of the group level spatial maps.

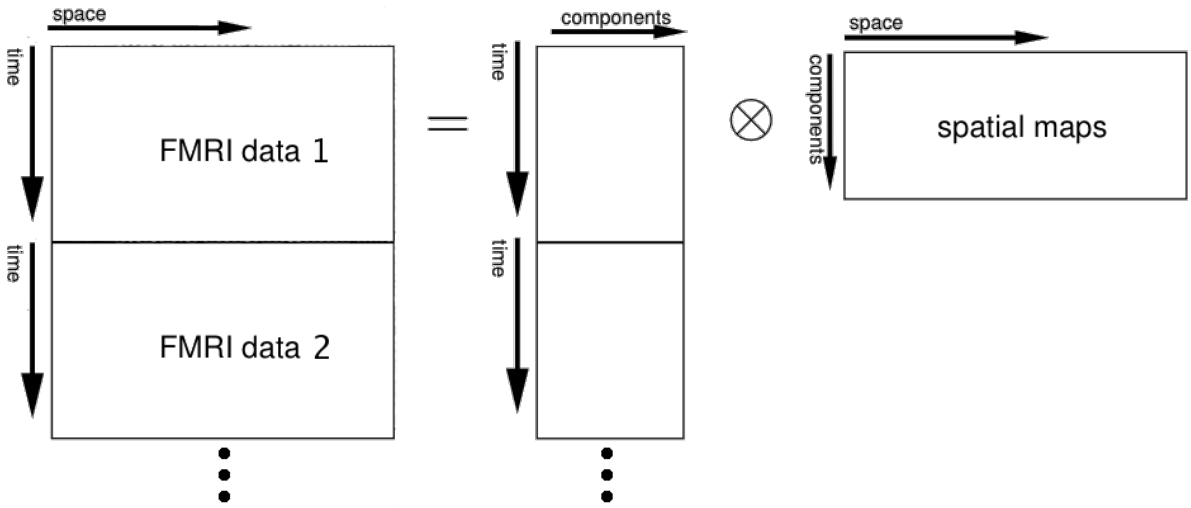

Figure 3. *Generation of group average independent component maps using MELODIC*. 4D rs-fMRI data from each subject are concatenated over subjects. The resulting matrix is decomposed into spatially independent components, (e.g. resting state networks). In order to generate subject specific versions of these components, dual regression is performed (see the main text). (Figure is adopted from http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC)

A second approach of generating comparable ICA components across subjects is implemented in the GIFT toolbox (Calhoun et al., 2001). GIFT rely on template matching of spatial components and back-reconstruction, and render comparable results as MELODIC (for further information on GIFT, see e.g. Schöpf et al., 2010).

In **study II**, we employed the dual regression approach as instantiated in MELODIC. One of our reasons behind this choice was that we aimed to replicate Napadow et al. (2010), that used MELODIC.

1.2.1.3 Regional homogeneity

Regional homogeneity (ReHo) is a measure that index the similarity of time courses of neighboring voxels. ReHo can thus be understood as a measure of local connectivity between a voxel and its nearest neighbors. ReHo calculations are normally performed on unsmoothed

data (which also is the case for our **study I**). The similarity between the time courses can be calculated using different statistics. Two common are the Kandels Coefficient of Concordance (KCC), and coherence. Both are implemented in the DPARSF toolbox (Chao-Gan et al., 2010). When directly compared, the coherence measure was deemed more sensitive for detecting differences between healthy and patient cohorts, as well as between different resting state conditions. The authors proposed that the superior sensitivity for the coherence measure over the KCC measure could be due to it being less susceptible to confounds in the phase delay between time courses. Although the specificity and sensitivity was not directly compared statistically, the authors conclude that the coherence measure typically is preferred (Liu et al., 2010). On the basis of this preliminary recommendation, we used the Cohe-ReHo

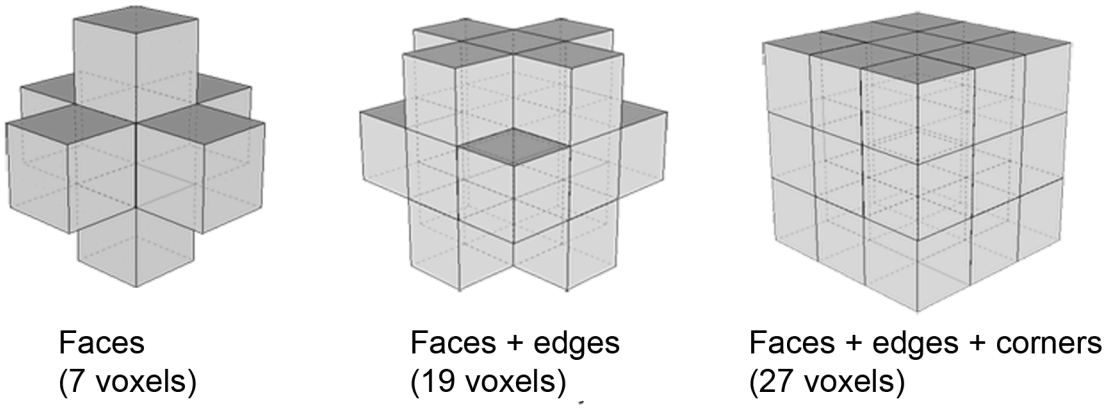

Figure 4. Cohe-ReHo is a measure of the average degree to which the BOLD signals in neighboring voxels covary, thus measuring local connectivity. In study I, neighboring voxels were defined as the voxels sharing faces, edges and corners (to the right). (Figure is adopted from http://fcp-indi.github.io/docs/user/reho.html)

Interpretations of the biological and functional significance of the ReHo measure primarily relies upon observations that ReHo are higher in regions overlapping with the default mode network and secondly, and that ReHo is higher in cortical grey matter regions (Liu et al., 2010). A theoretical justification of the ReHo measure is that intrinsic brain activity should be better reflected in clusters than single voxels. This voxel-wise measure is thus suitable for detecting local clusters with similar temporal activity profile, that subsequently could be used for SCA.

1.2.1.4 Graph theoretical network measures

Graph theory is a subfield in mathematics that was introduced by Leonard Euler in the 18th century. In rs-fMRI research, graphs are defined in terms of seed regions (aka. nodes or vertexes) and connections (edges) connecting the nodes. Network measures are used to characterize different properties of the network. Common network measures include degree centrality, cluster coefficient etc.

The properties of graph theoretical measures critically depend on the definition of the graph. That is, the to some extent arbitrary definition of nodes and connectivity thresholds used for

edge definition determine the results. By using weighed edges rather than binary, the challenge of edge definition is bypassed.

Arguable, a weakness of graph theoretical measures is that their neurophysiological validity is not always obvious. Considered that the BOLD-signal is an indirect measure, and that functional connectivity is inferred from this, graph theory resides on an even higher level of abstraction. However, the plethora of network measures is potentially a very valuable contribution to the toolbox of rs-fMRI measures. For instance, the possibility to reduce the rich spatiotemporal structure of the intrinsic brain activity to one (hopefully biologically justified) measure (such as those measuring the small-world-ness) offers a very condensed characterization of the functional connectome at a system level.

1.2.2 Spectral based measures

1.2.2.1 Fractional Amplitude of Low Frequency Fluctuations

Since the first publication of rs-fMRI (Biswal et al., 1995), oscillations of the BOLD signal within the frequency range between approximately 0.1 Hz to 0.01 Hz have been the primary focus of investigation. There are several reasons for this. Firstly, the power distribution of spontaneous oscillations in brain activity is biased towards lower frequencies. Low frequency fluctuations (<0.1 Hz) contributed to about 90% of the correlation coefficient between hubs of the same resting-state network (Cordes et al., 2001). Secondly, high frequency physiological noise (e.g. originating from cardiac – and respiratory movements) is less prevalent in the lower frequencies. Although there is support for the existence of functionally relevant oscillations at higher frequencies (up to 0.5 Hz, according to Chen et al., 2015), both theoretical and practical considerations typically lead researchers to use low pass band filters.

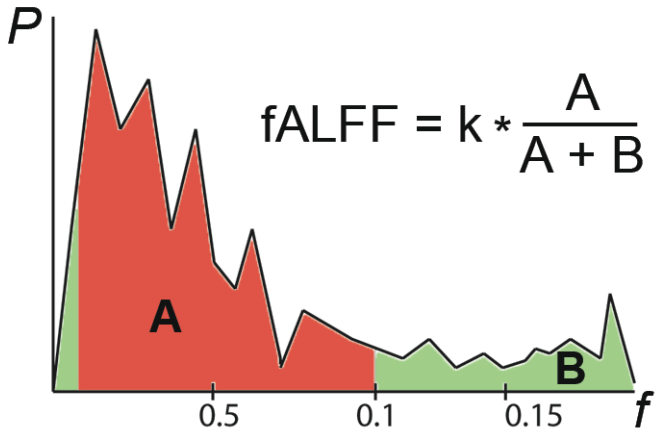

Figure 5. Fractional Amplitude of Low Frequency Fluctuations (fALFF) is a ratio of the power within a frequency range of interests (red) compared to the full spectral power (red and green). *P* denotes the power, and *f* denotes frequency, and k is proportionality constant.

Like the ReHo measure, fractional Amplitude of Low Frequency Fluctuations (fALFF) is a voxel-wise measure that does not require a priori specification of the localization of effects of interest, and thus it is less vulnerable to user imposed biases. In contrast to ReHo or other measures of functional connectivity, fALFF does not acknowledge the correspondence

between neighboring voxels time series (except indirectly, due to the spatial smoothing). fALFF is obtained by Fourier transformation of the time series that yields the power frequency distribution. Subsequently, the power of the frequency window of interest is calculated, and divided with the power of the total frequency band (Figure 6). The resulting measure reflects the degree to which the signal consists of lower frequencies compared to the full spectrum of frequencies. As with the ReHo measure, higher fALFF values are consistently observed within canonical resting state networks (particularly the DMN) and mainly restricted to grey matter (Zou et al., 2008).

1.2.3 Dynamic and forthcoming resting state measures

Functionally connectivity as described above is calculated by correlating the complete voxel time series (often ranging between 5-10 minutes). Thus, the resulting resting state network is a time average of the temporal correlations collapsed into one volume. Since many mental phenomena likely vary over a timescale of 5-10 min, their neural correlates would not be optimally captured using a connectivity average of the whole resting state session. The aim of dynamic rs-fMRI is to capture the fluctuations of intrinsic connectivity over time. One of the most common methods is the sliding window technique, where the time series is chopped up into smaller time series (or windows) of typically 20-60 time points. Thus, by "sliding" the window of the analysis one frame at a time, thorough out the full session, one calculates an (average) connectivity measure for each window. A drawback of this approach is that the connectivity measure of each time window is relatively unstable, since outliers in a time series of only 20-60 points greatly impact the connectivity measure (as compared to using the full time series consisting in the order of 200 time points). Secondly, the temporal resolution is only as good as the size of the time window. A repetition time (TR) of 2.5 and a window size of 30 time points render a temporal resolution of 75 s (which, however, certainly is an improvement compared to the duration of the whole session of about 5-10 min). Thirdly, since the frequency resolution from the fast Fourier transform of the time domain is inversely proportional to the number of sampling points, sliding window results in a more coarse spectral decomposition.

Another approach for investigating the time dynamics of intrinsic brain activity is temporal ICA. Temporal ICA detects temporal modes of brain activity, that in contrast to spatial ICA allow regional overlap (Smith et al., 2012). Temporal ICA rendered rather different networks than spatial ICA, but could, according to the authors, potentially offer greater biological interpretability.

Additional novel methods, such as point based connectivity measures (Thompson, in press; Karahanoğlu et al., 2015), and methods that reveals the frequency dependence of RSN and their interactions (Chang et al., 2010; Thompson et al., 2015), will further expand the methodological arsenal, and allow for a richer and hopefully more biologically grounded characterization of rs-fMRI.

1.3 METHODOLOGICAL CHALLENGES AND NUISANCES

A critical step and major challenge in rs-fMRI analysis is to control for non-neuronal noise in the BOLD-signal. In a minimally preprocessed (including realignment) dataset (N=20) acquired by high resolution 3T scanner within the human connectome project (HCP) (Glasser et al., 2013), it was estimated that on average, the variance associated with neuronal activity only constituted around 4% of the total variance of the BOLD-signal time series (Marcus et al., 2013). Motion and other non-neuronal factors explained most of the variance. Rs-fMRI is usually more vulnerable to non-neuronal noise than task based fMRI paradigms that rely on the subtraction methodology (section 1.1.1.1). This is because the confounding factors (e.g. head movement) that are common to both the experimental and the control condition are subtracted out. This is not the case in rs-fMRI connectivity analysis.

Linear regression is a common approach to control for noise in the BOLD-signal time series. Similarly to SCA (or task fMRI), the first step in nuisance regression consists in formulating a design matrix X, whose columns consist of factors to control for (e.g. movement realignment parameters and their time derivatives, signals form non-neuronal regions such as white matter and CSF etc.). The raw time series, Y, is to be explained by the nuisance regressors in X. The residual, or unexplained variance e, is the cleaned time series.

$$Y = X\beta + e$$

$$e = Y - X\beta$$

where

$$\beta = (X^T X)^{-1} X^T Y\,.$$

Using matlab, the β (the parameter estimates) can be calculated using the Moore-Penrose pseudoinverse of X ($pinv(X)$):

$$\beta = pinv(X) \cdot Y.$$

Thus, the cleaned data e (i.e. the data controlled for nuisance variables) is

$$e = Y - pinv(X) \cdot Y * X.$$

However, no control is perfect, and since the confounds (e.g. micro head-movements) are present in every dataset, there is no golden standard either with regard to what constitutes the ground truth of "clean" data, or regarding approaches of nuisance control. Fortunately, there is an ongoing development of more efficient and sophisticated strategies to control for BOLD variance of non-neuronal origin (Murphy et al., 2013), and promising avenues ranges from z-normalization (Yan et al., 2013) to ICA based approaches, such as the FIX (which is an acronym for FMRIB's ICA-based X de-noiseifier, Salimi-Khorshidi et al., 2014).

1.3.1 Head movement

As previously alluded to, movement is a major confound in resting state data. Recent investigations show that micro head-movement can cause spurious effects in brain connectivity measures that persist also after control for realignment parameters (Power et al., 2012). Head movements increase short distance-bilateral connectivity, and reduce long distance connectivity in the anterior posterior direction (Power et al., 2012; Van Dijk et al., 2012). Even after rigorous nuisance control, the effects of head motion affect resting state connectivity, why motion preferably should be matched across conditions/ groups with respect to the outcome of interest (Satterthwaite et al., 2013b).

1.3.2 Physiological confounds

In addition to the noise that is introduced by head movement and by the magnetic resonance (MR) hardware, there are several physiological sources of noise in the resting state data. Below I will review the more common physiological confounds.

Both respiration (Birn et al., 2006) and heart rate (Chang et al., 2009) contaminate rs-fMRI data, although predominantly at high frequencies that largely are filtered out using the conventional band pass filter of around 0.1-0.01 Hz. However, both cardiac and respiratory noise can be aliased into the lower frequency ranges when employing longer TR (2-3s) (Bhattacharyya et al., 2004). Moreover, pulse (Shmueli et al., 2007) and respiration rate fluctuates in the frequency range of conventional rs-fMRI (~0.03-0.04 Hz, Wise et al., 2004). These can manifest as spurious BOLD oscillations independent from (pulse and respiration induced) movement artifacts, by causing fluctuations in arterial CO_2 concentration and blood pressure (Wise et al., 2004).

CO_2 is potent vasodilator and hyperventilation decreases CO_2 levels in the blood, and decreases the BOLD signal correspondingly. Likewise, Haller et al. (2009) report that 45 seconds of breath holding (leading to hypercapnia), increases base-line BOLD signal by 2-3%, which is comparable to the amplitude of local task evoked responses. Using indirect measures of CO_2 concentrations, Wise et al. (2004) demonstrate that spontaneously occurring low frequency fluctuations (<0.05 Hz) of CO_2 concentrations lead to BOLD signal fluctuations in the order of ±0.1% in grey matter.

Many drugs affect the BOLD-signal amplitude. Several biochemical factors influence BOLD through vasoactive mechanisms that are unrelated to neuronal activity, as is the case for CO_2. Local decreases in BOLD response have been reported for nicotine (Giessing et al., 2006), cannabis (Jacobus et al., 2012) and ethanol (Luchtmann et al., 2013). Caffeine is vasoconstrictor, and can as such increase task evoked activity *relative* baseline (Mulderink et al., 2002). Others report that caffeine could either increase or have no impact on task evoked BOLD (Griffeth et al., 2011a), although caffeine has been associated with a large (4-6%) decrease in baseline BOLD signal (Griffeth et al., 2011b; Mulderink et al., 2002).

Additional confounding factors that influence the rs-fMRI data are inter-individual differences in functional anatomy and distribution of vasculature, which likely influence both amplitude and location of the hemodynamic response function (Frost et al., 2014). Not surprisingly, Handwerker and colleagues (2004) observed more variability of task-induced hemodynamic responses across subject than over different brain regions in the same individual.

1.3.3 Age

Age is another factor affecting the BOLD signal. Older age is associated with smaller BOLD amplitudes (e.g. D'Esposito et al., 1999; Tsvetanov et al., 2015). Tsvetanov and colleagues also observed decreased amplitudes of intrinsic BOLD-fluctuations (in rs-fMRI), which was taken as proxy for vascular function. When controlling for the reduced resting-state fluctuation amplitude, the age related reduced task evoked BOLD was only partially controlled for. This indicates that it is important to match or control for age at a group level. In the studies presented in this thesis, we have both matched groups with regard to age (**study I-IV**), and included age as a covariate of no interest in the group comparisons (**study II-IV**).

1.3.4 Anticorrelations and global signal regression

Global signal regression (GSR) is an effective measure for control of movement artifact in rs-fMRI data (Satterthwaite et al., 2013a; Yan et al., 2013). However, since GSR zero-center the distribution of correlation values, it typically induces spurious anti-correlations that are not present in the original data (Murphy et al., 2009). Therefore, alternative ways of controlling for non-neuronal signals that allows for investigation of anticorrelations have been proposed (Chai et al., 2011). A popular approach is the component based method, CompCor (Behzadi et al., 2007). In CompCor, principal component analysis (PCA) is performed on the time courses of the BOLD-signals within the white matter and CSF masks. The 3-5 first components pertaining to each tissue class is subsequently used as nuisance regressors at the subject level. The CompCor method was employed in the **studies II-IV**.

1.3.5 Low experimental control

The resting state is defined as an experimental condition where subjects are asked to abstain from any cognitive task. However, the resting condition is not a homogeneous phenomenal state, and critical voices have been raised regarding its interpretability (Morcom et al., 2007). Although the presence of spontaneous cognition is desired for certain studies (e.g. when researching mind-wandering), it can also introduce unwanted noise in the data. For instance, Tagliazucchi et al. (2014), reported that one third of a cohort of more than 1000 subjects drifted to sleep during a conventional resting state session. To prevent confounding effects from sleep, eye-tracking during eyes-open fMRI scanning could be used.

1.3.6 Replicability

The ability to replicate research finding is a prerequisite for scientific progression and a cornerstone in the scientific method. Several causes for lowered replicability in the biomedical research have been proposed. These include flexible designs, a high number of acceptable analytical approaches, strong publication pressure, and low statistical power (Ioannidis, 2005). Considered that the average power in neuroscientific studies has been estimated to 8%-31%, the latter factor is of critical importance (Button et al., 2013; David et al., 2013).

In an investigation of the reliability of rs-fMRI, Zuo and collegues (2010) reports moderate-to-high short (45min) - and long-term (5-6 months) test-retest reliability for resting state networks derived by ICA (using MELODIC). Wisner et al. (2013) reported an overall poor to good within-subject reliability of resting state networks. A review of task-based fMRI studies reports that the average longitudinal reliability measured as overlapping significant voxels were 30%, and the intraclass correlation coefficient (ICC) (one out of a vast set of existing reliability measures) was 0.50 (Bennett et al., 2010). Naturally, group differences and smaller effect sizes are harder to replicate than highly significant, strong within-subject effects.

Factors that hamper reproducibility of research findings across rs-fMRI studies are many, given the vast set of options of prepressing, statistical analyses, MR hardware and sequences, and scanning paradigms.

With regard to rs-fMRI design, Patriat et al. (2013) investigated the effect of task instruction (eyes open, eyes closed, fixation cross) on the degree of replicability of SCA probing six of the most common networks. Fixation cross rendered highest intra- and inter session ICC (except for visual and motor networks).

Also scan lengths impacts reliability. Birn et al. (2013) evaluated the effect of the durations of the resting state session on test–retest reliability of ROI-to-ROI connectivity (18 ROIs). Gains in intersession reliability began to diminish after 9–12 min, while improvements in intrasession reliability plateaued around 12–16 min.

Recently, a very laudable open science resource for establishing reliability and reproducibility in rs-fMRI was announced (Zuo et al., 2014). By providing more than 5000 resting state scans acquired at 18 international sites, from more than 1500 subjects, the Consortium for Reliability and Reproducibility (http://fcon_1000.projects.nitrc.org/indi/CoRR/html/) aims to catalyze development of new measures of reliability, and promote test-retest reliability as a minimum standard for rs-fMRI methods development.

Study II was partly an replication attempt of a study by Napadow et al. (2010), that we did not succeed to replicate. This highlighted the fact that group differences are subtle, demanding high-powered studies.

1.4 THE BIOLOGICAL BASIS AND PHYSICS OF THE BOLD-SIGNAL

Already in the early days of the rs-fMRI research, the biological origin and functional significance of rs-fMRI were questioned (Maldjian, 2001; Mitra et al., 1997). For instance, as reviewed in the previous section, several sources (e.g. head movement) of spurious resting state functional connectivity are known, supplying support for such criticism.

Naturally, any interpretation of rs-fMRI research heavily relies on what is known about the neurophysiological origin of the resting state brain signal. Many of the issues raised regarding the neural underpinning of the BOLD signal pertain as much to conventional task based fMRI as to rs-fMRI. For instance, without knowing precisely what neuronal activity (excitation, inhibition, subthreshold activity, bottom-up or top-down modulation) that is reflected in the changes of the BOLD signal, the functional interpretations will be very limited (Logothetis, 2008).

Compared to task evoked fMRI that possesses a relatively tight time-locking between the behavioral effect of interest and the BOLD response, the link between rs-fMRI and behavior is often more indirect. Thus, I believe it is particularly important to review the research that relate the resting state measures to the underlying biology and behavior in order to justify their use and understand their cognitive significance. Before I survey the evidence and proposals of the biological underpinnings and cognitive correlates of rs-fMRI, I will briefly review the physics underlying the MRI signal.

1.4.1 The physics behind the MRI signal

All rs-fMRI measures aim to quantify certain properties (the inter-regional covariance or the spectral characteristics) of the oscillating BOLD signal. The BOLD signal in turn is detected using suitable MR sequences that are sensitive to T2* weighted magnetic relaxation. The origin of the MRI signal relies ultimately on the MR physics. Thus, the physical foundation for the MRI signal poses a limit of what biological phenomena that can be captured using fMRI. The MR signal is generated from atomic nuclei possessing the quantum physical property of spin (a.k.a. angular momentum) that interacts with an external magnetic field and radio frequency pulses. Since around 80% of the body's atoms are hydrogen, MRI is particularly sensitive to imaging of the hydrogen nucleus. As a consequence, body parts that are rich in water, (i.e. CSF and soft tissues such as grey and white matter), yield stronger MRI signals, which makes these tissue classes bright in proton-weighted images. On the contrary, tissues low in water concentration and thus with low proton density, such as the bone in the scull and the sinuses, become darker. To record the nuclear magnetic resonance signal from the protons, the first step is to expose them to a strong magnetic field (typically 1.5- 3 Tesla), which causes the axis of the spin of the protons to either align (lower energy state,) or anti align (higher energy state). The differences in energy between the lower and higher energy states are small at room temperature, implying that the net magnetization that can be maintained at ordinary temperatures is small. Although the number of aligned protons, which constitute the basis of the MRI signal, barley outnumbers the anti-aligned protons, (with

around one proton per one million), the large number of protons makes this tiny fractional excess suffice to render a net magnetization in the direction of the external magnetic field (commonly refer to as the z-direction). The stronger the magnetic field, the larger the fraction of protons that gets aligned with it, and thus the larger net polarization. The external magnetic field causes the rotation axis of the protons to wobble with a frequency that is proportional to the field strength, according to the equation for Larmor frequency:

$$\omega_L = \gamma \times B_0$$

where ω_L is the angular frequency (the Larmor frequency), γ is the spin specific gyromagnetic ratio and B_0 is the magnitude of the external or applied magnetic field. The Larmor effect is a crucial phenomenon underlying both signal generation and signal localization. Due to the relationship between angular frequency and the magnetic field, selective excitation of protons, localization of the signal, and contrast mechanism used to retrieve biologically significant information are possible.

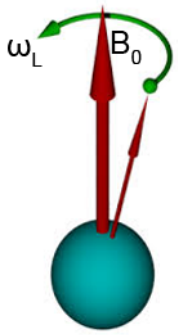

Figure 7.The Larmor equation describes the frequency $\boldsymbol{\omega_L}$ by which the spin axis rotates in the presence of an external magnetic field $\boldsymbol{B_0}$.6

The aligned protons can be excited to the higher energy state by means of a radio pulse that has a frequency that matches the Larmor frequency. By applying an additional magnetic gradient along the z-direction one can selectively excite the protons in a plane perpendicular to the z-axis. When the protons absorb energy from the radio pulse, their direction of procession (or axis of spin) swaps 180 degrees. The net magnetization caused by the applied radio pulse varies between 0 and 90 degrees (the so called flip angle) for a gradient echo sequences. After the radio frequency transmitter has been turned off, relaxation happens. Protons that absorbed energy will spontaneously return to the lower energy state. During the relaxation process, the absorbed energy is emitted as radio frequency radiation. The receiver coil (commonly with 8 or 32 channels) detects the energy that is emitted during relaxation. To localize the signal within the x-y plane (that is perpendicular to the z-axis), a transient phase encoding gradient is applied, rendering different phases typically along the y-direction (i.e. in the anterior-posterior direction of the brain). Finally, to localize the signal in the x-direction (in the left to right direction), a frequency-encoding gradient is applied throughout the data recording of that slice. Thus, each location in the x-y plane is marked with a unique combination of phase and frequency at a given time of signal recording, allowing for a 2D image reconstruction through Fourier transformation of the MR signal.

1.4.1.1 The MR contrast -T1 and T2* weighted images

By varying the onset times and durations of the three spatial gradients (i.e. the slice selection, frequency encoding and phase encoding gradients), the excitatory radio frequency pulse and the time period between excitation and signal read out (time to echo, TE), one can define a wide range of different MR sequences. Ingenious set up of the MR sequences allows for imaging certain aspects of the investigated object. In current thesis, two kinds of sequences

are used. The first is for T1 weighted images that yields high spatial resolution and demarks different tissue classes. The second is echo planar imaging (EPI), resulting in T2* weighted images suitable for fast imaging of the blood level dependent (BOLD) signal.

The MR camera only detects transverse netmagnetization. After application of the 90 degree pulse, net magnetization is in the transversal plane, and the (indirectly inferred) longitudinal magnetization is zero. Once the 90 degree pulse is turned off, the relaxation occurs, whereby the absorbed energy is emitted by the protons. During the relaxation period the longitudinal magnetization recovers, and the transvers magnetization decays. The decay of the transverse magnetization following the RF pulse is a sinusoidal with an exponentially decreasing amplitude envelope, called Free Induction Decay (FID). The rate of the FID is described by the time constant T2*, and is determined by two factors. First, the tissue specific spin-spin relaxation caused by random interaction among protons, where the microscopic magnetic fields generated by the spinning protons cancel out each other. The tissue specific time-constant describing the decay of transversal magnetization due to spin-spin interactions is called T2. The second factor driving FID is static inhomogeneities in the magnetic field causing dephasing of the proton. By applying a 180-degree RF pulse during image acquisition, spin dephasing due to static inhomogeneities in the magnetic field are reversed. This allows for detection of the tissue specificity in spin-spin relaxations, rendering T2 weighted images. The T2* is thus always smaller than T2. (For an introduction to MR-physics, see e.g. Pooley, 2005)

The BOLD signal is recorded using the T2* weighed MR-sequences. In the studies reported here, we have employed echo-planar imaging (EPI). As traditionally used for T2* weighted imaging, a long TR (2.5s) was used which allow for restoration of longitudinal magnetization. The time to echo (TE) was 30 ms, so sufficient T2*-dephasing would have time to occur.

Like the T2, the time describing longitudinal magnetization recovery, T1, is tissue specific. It is determined by the spin-lattice relaxation. The thermal motions of the protons within their molecular structure causes a magnetic field called the lattice field. When excited protons interact with the surrounding nuclei in lower energy states, their energy is dissipated and increases the temperature of their immediate environment. In this way, the energy absorbed by the protons during the RF pulse is transferred to the surrounding lattice, and the proton returns to its lower energy state. This causes the net longitudinal magnetization to increase. Hence the time constant T1 denotes the time required for the netmagnetisation to be restore to 63% of its original value in the z-direction after being flipped into the transverse plane by the 90 degree pulse.

1.4.2 BOLD contrast mechanisms

Local changes in concentration of deoxyhemoglobin alters the BOLD signal intensity (a principle first described by Ogawa et al. 1990). Deoxygenated blood is more magnetic (paramagnetic) than oxygenated blood, which is almost diamagnetic and therefore unaffected

by an external magnetic field. Consequently, oxygenated blood does not cause perturbations (i.e inhomogeneities) in the local magnetic field surrounding the blood vessels, whereas deoxygenated blood does. As mentioned above, static inhomogeneities in the local magnetic field cause spin dephasing and signal loss in T2* (BOLD) weighted images.

What governs the oxygen level in the blood in the brain? Three predominant factors have been identified (for a detailed review, see Kim et al., 2012). The most influential is cerebral blood flow (CBF). Blood flow washes out deoxygenated hemoglobin and causes an increased BOLD signal. A second factor is brain activity induced changes in cerebral blood volume (CBV). This leads to increased amount of deoxyheaemoglobin and thus a signal decrease. Finally, neuronal activations causes increased cerebral metabolic rate of oxygen consumption ($CMRO_2$), causing deoxyhemoglobin to increase and the BOLD signal to decrease. The canonical hemodynamic response function is modeled as a small initial dip in BOLD signal following neuronal activation, followed by an increased BOLD response constituting the main BOLD response, and ending with a post-stimulus undershoot. The initial dip is believed to be driven by the increased oxygen consumption, whereas the main response likely is driven by increase in the blood flow (Buxton, 2010) (see the section on neurovascular coupling, 1.4.2.1). The mechanisms driving the post-stimulus undershoot is poorly understood. To the extent that the initial dip is detectable, it could theoretically serve as a more exact reflection of neuronal activation (in terms of both temporal and spatial proximity to the underlying activation). However, in practice, due to the limited signal to noise ratio in fMRI, the signal increase of the main BOLD response that peaks at 5-10 seconds subsequent to the triggering brain activity dominates. Neural activity can cause local increases in CBF of around 50%, and a 30% increase in oxygen consumption. This leads to an increase of the BOLD signal amplitude by a few percent (Griffeth et al., 2011b). Task induced BOLD-signal changes are in the order of 0.5-1% for cognitive tasks (Desmond et al., 2002), and seldom above a few percent (< 5%) for motor tasks which generally yield the largest and most robust BOLD responses (see e.g. Rao et al. 1996).

1.4.2.1 Neurovascular coupling – relating neuronal activity and blood flow

Knowing the mechanisms by which neuronal activity causes increased blood flow (CBF) is crucial for understanding the neuronal basis of the BOLD signal. Several different mechanisms governing neurovascular coupling have been proposed, and different factors likely play a role. Neuronal activity refers to different cellular processes on different spatial-temporal scales. Adapting a reductionistic approach, a first step would be to define the smallest unit of neuronal processes that play a role for cognition. Pragmatically, neuronal spiking or action potentials are considered the “atoms” of the neuronal substrate supporting information processing. Similarly to how a huge complexity and diverse set of functions can be instantiated through simple on-off switches (transistors) that make up computers, the binary signaling of action potentials could constitute the smallest unit of neuronal computing. However, it is possible that neurons are more complex in their functionality than only integrating input to render binary output signals. Examples of biological processes supporting

information processing in the absence of action potentials are neuronal gap junctions and glia cell activity (Perea et al., 2005). An argument for sub-cellular information processing is the fact that single cell organisms like amoebas could carry out complex behavior such as to swim, eat and multiply in the absence of any action potentials or synaptic connections, (Hameroff, 2014). The research on what constitute the ultimate, atomic unit of biologically generated information processing will certainly affect the field of cognitive science, and possibly force us to redefine the notions of neuronal activity.

For the purpose of understanding neurovascular coupling, these fundamental questions on what neuronal activity to map cognitive processes upon will be suspended for now. Instead it suffices to state that a proxy for neuronal activity is energy consumption. A dominating consumer of energy is the post synaptic sodium-potassium (Na^+/K^+) channels, that help recovery from post synaptic excitatory activity by restoring high intracellular potassium and extracellular sodium concentrations. Attwell et al. (2002) estimate that these postsynaptic ion pumps require about 75% of the brains energy consumption. Additionally, other ion pumps, action potentials and the recycling of neurotransmitters require energy. Energy is primarily obtained from adenosine triphosphate (ATP), that is synthesized either anaerobically through glycolysis, or aerobically produced through oxidative glucose metabolism. The aerobic ATP production is the dominant, responsible for around 90 % or the ATP production (Pasley et al., 2008). Both the glucose and oxygen is supplied by the blood, which also helps to remove metabolic waste products. Although correlated, the mechanistic relationship between increased metabolic demand and CBF is still not well known. There are however a couple of different models, each likely describing parallel mechanisms. One is that CBF is directly controlled by energy demand. In this view, metabolic bi-products, such as nitric oxide, adenosine, CO_2, potassium and arachidonic acid act, either directly or via mediators, on the smooth muscles of the arterioles. These feedback mechanisms could either be vasoconstructive or vasodilative, thus down- or up regulate CBF. Alternatively, models of so called feed forward mechanisms stipulate that the release of neurotransmitter induces a saccade of biochemical events in the surrounding astrocytes and glia cells, which ultimately have a vasoregulatory effect on the blood vessels (Pasley et al., 2008).

The BOLD signal change is thus due to a complex function of the change in CBF, the baseline deoxyhemoglobin levels CBV, and the balance between changes in CBF and $CMRO_2$ (Buxton, 2010).

1.4.2.2 Content of a voxel

The smallest 3D units of MR-images are voxels. In most of the projects presented here (**study II-IV**), the MR images are resamples to 2x2x2 mm^3 voxels. Given that a gross estimate of the average number of neurons per mm^3 cortical tissue is in the order of 25 000 (several times higher in e.g. visual areas, and lower in motor areas) (Logothetis, 2008), each of our unsmoothed voxels thus contains about 200 000 neurons. Each voxel also contains 3.2 km of dendrites, 32 km of axons and $4\text{-}8\times10^9$ synapses. (Corresponding numbers for 55 μl grey matter, which was taken as a representative voxel sizes in the literature until 2008, were 5.5

million neurons, 2.2-5.53×10^10 synapses, 22 km of dendrites and 220 km of axon, according to Logothetis, 2008). After applying spatial smoothing, the number of constituting neuronal elements contributing to a voxel is further increased.

''It makes no sense to read a newspaper with a microscope'' – the neuroanatomist Braitenberg

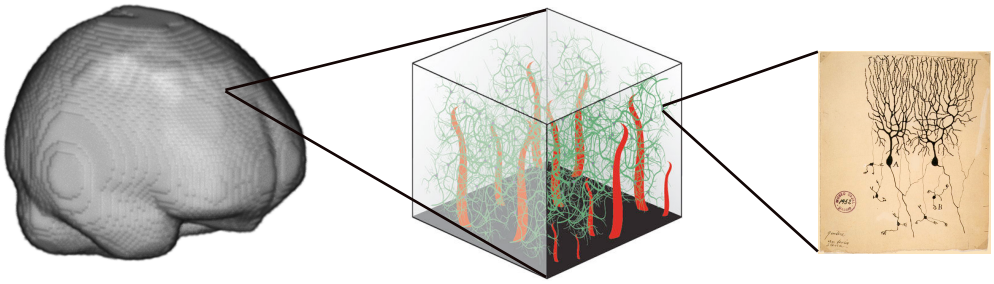

Figure 7. Typical spatial resolution for fMRI. A solid a priori brain mask with a voxel size of 2x2x2 supplied by SPM (far left) consists of around 250 000 voxels. Each gray matter voxel contains 200 000 neurons (cf. 8.6 ×10^10 neurons in the whole brain, of which the majority are found in cerebellum and only approximately ¼ resides in cerebral cortex) (Azevedo et al., 2009; Herculano-Houzel, 2009). Figure is based on adjustments of Logothetis (2008) and https://upload.wikimedia.org/wikipedia/commons/1/15/PurkinjeCell.jpg .

1.4.3 BOLD and electrophysiology

As mentioned above, a popular proxy for neuronal activity is neuronal spiking. Common electrophysiological methods include multi-unit spiking activity (MUA), local field potentials (LFP), and at the system level -electro encephalography (EEG) and magnetic encephalography (MEG). Multi-unit spiking activity is recorded by placing a micro-electrode away from spike-generating sources so that a single action potential does not dominate the signal. By applying a high pass filter of 300-400 Hz, it its believed that MUA signal reflects the simultaneous spiking of thousands of neurons, reflecting the spiking output of a cortical area (Heeger et al., 2002). Local field potentials uses similar set-up as the MUA, but instead of high-pass filtering a low-pas filter of around 300 Hz is used (Logothetis, 2008). When synaptic inputs are synchronous, the resulting extracellular currents around the dendrites add up and increase the LFP. LFP is believed to particularly reflect input to a cortical area as well as local intra-cortical processing, rather than neuronal output. Seminal work on the relationship between electrophysiology and the BOLD signal suggests that BOLD predominantly is associated with the amplitude of LFP (Logothetis et al., 2001). Moreover, the BOLD signal does not seem to be causally linked to neuronal spiking. Mathiesen and colleagues (1998) simultaneously recorded LFP, spiking activity and CBF in the rat cerebellum, while modulating the spiking activity of the Purkinje cells by stimulating inhibitory interneurons. Stimulating inhibitory interneurons decreased the spiking of the Purkinje cells, whereas both the LFP and the CBF increased. Thus, the current opinion is that the BOLD signal most closely reflects input and intra-cortical processing, rather the spiking output.

1.5 THE BIOLOGICAL AND FUNCTIONAL SIGNIFICANCE OF RS-FMRI

The extent to which the low frequency fluctuations of rs-fMRI signals directly reflect BOLD-signal fluctuations of neuronal origin or are due to low-frequency artifacts, certainly matters for the interpretation of rs-fMRI. There are ample evidence on how head movement (Power et al., 2012), cardiac artifacts (Shmueli et al., 2007) and the preprocessing strategies (Bright et al., 2015) influence the resting state results. As much as 96 % of the variance of in the resting state fMRI time series could be explained by non-neuronal factors (e.g. physiological artifacts, head movement and scanner noise), (Marcus et al., 2013). This has early on provoked a healthy skepticism regarding the neurophysiological significance of resting state networks (Maldjian 2001).

1.5.1 Six sources of support that rs-fMRI reflects neural processes

Below I account for findings that convincingly show that resting state networks (at least partly) reflect neuronal activity.

1.5.1.1 Support from callosotomy

Johnston et al. (2008) compared intrahemispheric functional resting-state connectivity before and after a sectioning of the corpus callosum in an epileptic child. Using seed regions in five bilateral brain regions (in frontal eye field, lateral parietal-, primary visual-, hippocampal-, and somatomotor cortex), they observed a striking reduction in intrahemispheric functional connectivity, while interhemispheric connectivity patterns largely remained unaffected. Thus, this unique data strongly support the notion that resting state functional connectivity reflects neural communication that requires anatomical connections.

Similar conclusions was reached Quigley and colleagues (2003), investigating three subjects with agenesis of corpus callosum. The congenital callosal malformation was associated with disrupted intrahemispheric functional connectivity in motor and auditory networks, compared to healthy controls. In contrast however, Tyszka et al. (2011) observed intact bilateral RSN despite absent corpus callosum among eight subjects, suggesting that RSN could emerge flexibly with the development of normal cognition and behavior.

1.5.1.2 Support from electrophysiological correlates

There are several studies relating slow oscillatory rs-fMRI with electrophysiology. For instance, in a electrocorticograpical (ECoG) investigation of five epileptic patients, He et al. (2008) conclude that the ECoG signals (below <4 Hz, and high gamma between 50-100 Hz) displayed similar spatial correlational structure as BOLD (although not acquired simultaneously). Hiltunen et al. (2014) performed concurrent EEG and fMRI during a resting condition, and correlated the ICs obtained from infraslow EEG (0.01-0.1 Hz) with those obtained from rs-fMRI. They concluded that infra-slow EEG fluctuations were correlated with intrinsic BOLD signal fluctuations in brain regions corresponding to several of the canonical fMRI RSN. Using MEG, Brookes and colleagues (2011) performed both ICA and SCA on the amplitude envelope of source-space neural oscillatory signals. They observed a

similarity of rs-fMRI connectivity and the connectivity pattern generated from the MEG β-band (13-30 Hz) amplitude envelope.

Although these studies provides initial evidence of electrophysiological correlates of rs-fMRI, much work remains to establish e.g. what frequency bands of each recording modality that best corresponds to the endogenous BOLD signal.

1.5.1.3 Support from signal localization in grey matter

In an attempt to identify the anatomical underpinning of resting state networks, De Luca and colleagues (2006) performed ICA analysis in relative high resolution (in plane resolution 2x2 mm^2) resting state EPI data. By manually delineating the boarder between gray and white matter, they were able to localize the resting state BOLD-signal primarily to the grey matter regions, rather than to white matter or CSF.

1.5.1.4 Support from anatomical connectivity

There are several multimodal MR studies confirming overlap between functional and structural connectivity. Greicius et al. (2009) investigated the default mode network by both rs-fMRI and diffusion tensor imaging (DTI) in six healthy participants. Choosing seed regions in the posterior cingulate cortex (PCC) for both analyses, they observed monosynaptic connections between PCC and hippocampal formations, whereas no such were found between hippocampus and medial prefrontal cortex. The results are interesting for two reasons. Firstly, it underscore that within DMN functional connectivity has monosynaptic connection counterparts, as revealed by DTI. Secondly, it shows that functional connectivity is not limited to monosynaptic linkage, but also reflects polysynaptic connections.

There are now multiple studies reporting that structural and functional connectivity is highly interrelated (Hagmann et al., 2008; Koch et al., 2002). Using the more sensitive (compared to DTI) diffusion spectrum imaging (DSI), Honey et al. (2009) conducted computational modeling of rs-fMRI and DSI data, based on recordings from five subject. Their empirical and modeling results demonstrated that although resting state functional connectivity was variable and frequently present between regions without monosynaptic structural connections, its strength, persistence, and spatial distribution was constrained by the structural connectivity. Similarly, Goñi and colleagues (2014) based a computational model on anatomical connectivity measures that strongly predicted measures of functional connectivity, leading them to conclude that functional connectivity largely is shaped by anatomical networks.

1.5.1.5 Support from BOLD-like properties– vascular density, TE-dependence and response to CO_2

CO_2 is a potent vasodilator causing increased blood flow and thus increased BOLD-signal. Similarly to how CO_2 modulates task evoked fMRI effects, Chang et al. (2009) reports that

naturally occurring fluctuations of CO_2 (measured using e.g. a breathing belt), significantly predicted resting state oscillations.

The amplitude of intrinsic BOLD-signal oscillations (such as fALFF) has been related to interregional differences in vascular density in a similar way that task-induce BOLD-signal has (Vigneau-Roy et al., 2014). By relating the segmentation output (grey matter, white matter and vascular density) to BOLD response, they found that the correlation of regional BOLD amplitude (both in resting state and task fMRI) was higher to vascular density maps than to grey matter density maps.

Finally, there is evidence that intrinsic BOLD-signal display similar linear relationship to the echo-time (TE) as task evoked fMRI does. The BOLD contrast (not signal) is thought to linearly increase with increasing TE (Menon et al., 1993). Drawing on this property of the BOLD contrast, Peltier et al. (2002) showed a linear relationship between TE task induced BOLD response that also was found for resting state connectivity. Similarly, by performing ICA on rs-fMRI data, Kundu et al. (2012) convincingly demonstrated differentiations between typical noise-related ICs (e.g. due to movement) and neuronal resting state networks (e.g. the DMN), based on TE-dependence. That is, they were able to demonstrate that neuronally plausible ICs displayed TE dependence similarly to task related fMRI contrasts, whereas non-neuronal components did not. Taken together, these studies support the notion that the same neurovascular BOLD-mechanisms observed in conventional task based fMRI contributes to rs-fMRI.

1.5.1.6 Distinct resting-state networks bear similarities to task activation maps

Not only do the dozen or so of the most commonly reported resting state networks visually appear to reflect a plausible functional organization of the brain (such as the sensorimotor-, visual-, auditory-, frontoparietal networks etc.). Systematic comparisons between thousands of group activation maps (listed in the BrainMap database, http://www.brainmap.org/) and resting state networks (from ICA) revealed correspondences, leading the authors to conclude that the "*full repertoire of functional networks utilized by the brain in action is continuously and dynamically "active" even when at "rest"* " (Smith et al., 2009, p.1).

1.5.2 Cognitive and biological functions

Above we have reviewed evidence that rs-fMRI (i) typically is confounded by several factors (particularly head movement), and (ii) have a neuronal basis, as confirmed by a wide range of different approaches that were listed in previous section. The natural follow-up question is, what biological and functional roles does the intrinsic brain activity have? This is a crucial question that has received well deserved attention. Although much work remains to be done, several functions have been stipulated. In the following I aim to give an overview of the most frequently proposed roles and survey some empirical findings supporting them.

1.5.2.1 Intrinsic activity modulates behavior

Models of the brain as a "prediction machine" or "bayesian brain" describe a likely fundamental neuroscientific principle (Friston, 2013; Ma et al., 2014). For instance, we know that only a small fraction of the sensory data that reaches sensory organs (e.g. the retina) arrives at their final destination in the brain. Still, a rich, multimodal phenomenal experience is generated. Based on this, complex behavioral responses are prepared. A large part of the information processing by the brain is simulations and predictions. Consistent with the bayesian brain model, a major function of the metabolically expensive intrinsic brain activity could be to integrate past experiences, simulate and predict future events, and facilitate behavioral responses. A considerable number of studies demonstrate how intrinsic brain activity influence overt behavior (for nice reviews, see Northoff et al., 2010; Sadaghiani et al., 2013). To mention just a few, Fox and colleagues (2007) demonstrated how intrinsic fluctuations in the motor cortex explained 74 % of the trial-by-trial variance in grip force. In the cognitive domain, Hesselmann et al., (2008) showed how the ongoing activity in the fusiform area during the prestimulus interval (20-50s) prior to presentations of Rubin's ambiguous vase–faces figure, biased perception of face over the vase. Recently, employing a similar strategy, Sadaghiani and colleagues (2015) tested how the whole brain functional connectivity during the prestimulus interval affected performance on a continuous auditory detection task. The authors report that before misses, connectivity showed reduced within network modularity, indicative of more random and less organized connectivity patterns across brain regions. Intrinsic brain activity in task relevant regions also predicts pain intensity perception (Boly et al., 2007), attentional lapses (Weissman et al., 2006), and response inhibition (Mennes et al., 2011). Thus, it seems likely that the endogenous brain activity "keeps the motor running", and can facilitate task-relevant actions.

1.5.2.2 Intrinsic activity is modulated by behavior

Not only do intrinsic brain activity influence behavioral performance, but it also display plasticity in response to training and experience (for a review, see Guerra-Carrillo et al., 2014).

Ma et al. (2011) reported an increase in resting state functional connectivity between motor-related areas following 4 weeks of daily finger movement training. Similarly, Taubert et al. (2011) reported resting state connectivity changes following two sessions of balance training. Further changes were observed during an extended practice for 6 weeks, which were related to behavioral improvements. In shorter time scales, training induced connectivity changes involving task related areas have been observed already after 5 min of language production (Waites et al., 2005). These and many other similar investigations demonstrate that resting state activity is influenced by experience. Consequently, rs-fMRI is also vulnerable to spill-over effects from task paradigms preceding rs-fMRI scanning (Stevens et al., 2010; Wang et al., 2012), which bear relevance to all **studies (I-IV**) constituting the current thesis. Importantly, these finding support the notion that resting state functional connectivity reflects

a repeated history of co-activation among distributed brain regions. Thus, the 'Hebbian' saying "fire together –wire together" seems valid also at the system level.

1.5.2.3 Non-conscious household function

Resting state networks exists during task (Cole et al., 2014; Fransson, 2006), in sleep (Horovitz et al., 2009), coma (Boly et al., 2008), and in other species such as rodents (Hutchison et al., 2010). Hence, it is reasonable that the role of intrinsic brain activity is not exclusively related to conscious mental processing. Rather, intrinsic brain activity likely contributes to maintaining the physiological health of the brain. Such "housekeeping" roles could include neuronal repair, restoration of ion gradients and protein trafficking (Raichle et al., 2006). These and other homeostatic mechanisms are necessary to ensure stable neuronal and network functions (Marder et al., 2006). However, the exact role of intrinsic brain activity for maintaining the brains homeostasis is currently not well understood (Raichle et al., 2006).

1.6 RS-FMRI APPLIED

As previously alluded to, intrinsic brain activity is structured at different temporal scales. On one hand, resting state networks reflects phylogenetically old principles of brain organization. Homological counterparts of resting state networks can be found even in rodents (Hutchison et al., 2010). At the scale of the human lifespan, the resting state networks are to some extent at place already at early childhood, and undergo developmental changes throughout the lifespan (Betzel et al., 2014; Fransson et al., 2007). Rs-fMRI mirrors structural brain connectivity, that is reasonably stable over the lifespan. Abnormal rs-fMRI has been found in more than 20 pathological conditions persisting from months to many years (Fox et al., 2010). At even smaller timescales, long term (several months, Taubert et al., 2011), midterm (a couple of days, Sami et al., 2014) and short term (as short as a few minutes, Waites et al., 2005) interventions impacts intrinsic brain activity. The malleability of rs-fMRI due to exposure to endogenous and environmental conditions makes it a potentially useful measure for phenotyping. Mapping group differences could improve our understanding of the functional abnormalities causing disease. Eventually, the hope is that rs-fMRI biomarkers could be applied at the level of single individuals to aid (presymptomatic) diagnosis and predict treatment response. Additionally, reliable characterizations of pathological rs-fMRI could be used to identify new drug targets and treatments.

Currently however, the clinical applications of fMRI in general, and rs-fMRI in particular, are sparse. The primary clinical use of fMRI worldwide is pre-surgical mapping (Matthews et al., 2006a). For this, rs-fMRI has proven to be a valuable complement to task based fMRI (for a review, see Lang et al., 2014). The next area of application for rs-fMRI would likely be to aid diagnosis, where studies already report high sensitivity and specificity for rs-fMRI based classifications of conditions such as Alzheimer (Thomas et al., 2014). Further, putative applications are predicting treatment outcome that could help inform decision making regarding suitable treatments. In basic neuroscience, rs-fMRI continues to provide an

important tool to understand the etiology of psychiatric disease and the mechanisms by which treatments restore pathological brain processes. However, an immutable prerequisite for any application of rs-fMRI is high methodological reliability, regardless if used in clinical, juridical or educational contexts.

1.6.1 Resting state and pharmacological fMRI

Pharmacological fMRI refers to the applications of fMRI methods for direct or indirect measures of drug action. Pharmacological fMRI could potentially provide a useful tool for drug development, particularly during the early phases (see Matthews et al., 2006b; Wise et al., 2006). However, a concern when interpreting the results of any pharmacological fMRI study is the possibility that an altered BOLD response could be due to hemodynamic effects, rather than drug-induced changes in neuronal activity per se (Iannetti et al., 2007, see also the discussion in section 1.3.2). Theoretically, both amplitude and timing of the hemodynamic response could be affected. For instance, Hirano et al. (2008) used PET imaging to dissociate metabolic and neurovascular effects of L-dopa treatment among Parkinson patients. In the context of functional connectivity analysis, the strength of the (Pearson) correlation coefficients should in principle be unaffected by drug related changes in signal amplitude, since correlation merely is a measure of linearity. However, due to the low signal-to-noise ratio in EPI data, amplitude would play a role. Moreover, potential pharmacologically induced phase difference of the BOLD signal in separated brain regions would unavoidably affect the measured functional connectivity.

In **study I**, two different psychoactive agents (L-dopa and benzodiazepines) were investigated using identical methodology and statistical analyses. One advantage of investigating two different drugs in the same study is that the specificity of the drug actions could be estimated. For instance, resting state networks that a priori were hypothesized to primarily be affected by oxazepam could serve as control networks when examining the effects of L-dopa, and vice versa (in accordance with the guidelines advocated by Fox et al., (2010).

1.6.1.1 L-dopa

L-dopa is a precursor to dopamine, which in contrast to dopamine crosses the blood-brain barrier. Hence it can be used as a centrally active dopaminergic drug, and as such it is primarily used against Parkinsonism. Dopamine is a key neurotransmittor and plays a central role in motor control, reward-driven behavior, working memory and cognition. Dopamine receptors are distributed throughout the brain, with the highest densities of the most common types (DA1 and DA2) in the striatum and the brainstem (Volkow et al., 1996). Depending on the target receptor, dopamine is either excitatory or inhibitory. An important study by Kelly and colleagues investigated the effects of L-dopa on resting-state activity in healthy subjects. Their main findings were increased connectivity between putamen and the cerebellum, between the nucleus accumbens and the ventrolateral prefrontal cortex, as well as a decreased connectivity between the caudate nucleus and the DMN (Kelly et al., 2009). However, their

study was limited in scope to only detect changes in connectivity between prespecified regions in the striatum and the rest of the brain. In an attempt to extend upon their results, we conducted **study I.**

1.6.1.2 Benzodiazepine

Oxazepam is a benzodiazepine that acts inhibitory on brain activity. It is a commonly prescribed anxiolytic, and is also used to help people staying asleep. Benzodiazepine exerts its influence by binding to $GABA_A$ receptors that, upon activation, conducts Cl^--ions through its pore, resulting in hyperpolarization of the neuron. This causes an inhibitory effect on neurotransmission by diminishing the chance of a successful action potential to occur postsynaptically. $GABA_A$ receptors are distributed all over the cortex, but with higher concentrations in the occipital cortex and the anterior cingulate cortex (ACC) (Abadie et al., 1992; Cameron et al., 2007). The effects of benzodiazepine (midazolam)-induced sedation on rs-fMRI connectivity have in a previous study mainly been associated with increased connectivity in motor and visual areas, and decreased connectivity in the default mode network (DMN) (Greicius et al., 2008). However, the rs-fMRI effects from small, non-sedative doses of benzodiazepines that are routinely administered to patients suffering from anxiety disorders are not well studied.

1.6.2 Resting state imaging of rheumatic pain

Potentially, rs-fMRI could be used for numerous clinical purposes, such as diagnosis and prediction of treatment response (Fox et al., 2010). In the current thesis we present two attempts to characterize the endogenous brain activity associated with rheumatic pain; in fibromyalgia (FM) and in rheumatic arthritis (RA). Additionally, we also evaluate the effects of physical exercise in FM, and the putative normalizing effects of exercise on rs-fMRI.

According to Hardin (1990), rheumatic symptoms are characterized by:

"(1) pain or discomfort, usually perceived in the vicinity of one or more joints (including the spine); (2) pain on motion of the affected area(s); (3) soreness (to the touch) of the affected region(s); (4) stiffness of the affected part(s), especially after a period of immobility; (5) symptomatic improvement after mild exercise, but worsening after vigorous exercise; (6) symptomatic worsening in response to climatic factors, especially falling barometric pressure and rising humidity; and (7) symptomatic improvement in response to warming the affected area(s). Not all rheumatic pain syndromes have all seven characteristics, but most will at least have the first four."

1.6.2.1 FM

Fibromyalgia (FM) is a condition characterized by widespread, long-lasting pain, a lowered pain threshold (allodynia), as well as an increased sensitivity for suprathreshold pain (hyperalgesia). FM afflicts about 2% of the population, of which almost 90% are women (Wolfe et al., 1995). In addition to pain, commonly occurring symptoms include cognitive

dysfunctions, fatigue, and sleep disturbances, and there are also known comorbidities with conditions such as depression (Weir et al., 2006). The polysymptomatic nature of FM leads to great suffering for FM patients and high costs for society. Unfortunately, current treatments are not very efficient (Carville et al., 2008). Drugs (antidepressants such as selective serotonin reuptake inhibitors and serotonin noradrenaline reuptake inhibitors), education in pain coping strategies, and physical exercise (as investigated in study III) belongs to the more commonly ordinated treatments (Clauw, 2014).

Little is known about the neurophysiological mechanisms mediating the effects of physical exercise treatment on pain reduction. Peripherally, exercise could restore a locally disturbed micrometabolism (Mannerkorpi et al., 2003), reduce levels of proinflammatory markers (Ortega et al., 2009) and increase growth factors IGF-1 (Bjersing et al., 2013). Centrally however, few if any study has investigated effects of physical exercise on chronic pain in FM.

In the papers included in the thesis (**study II** and **III**), FM is classified based on criteria by American College of Rheumatology (ACR) from 1990 (Wolfe et al., 1990). These include widespread bodily pain that has been ongoing for more than 3 months that cannot be explained by other diseases. Although much remains to be understood regarding the neuronal mechanisms that are involved in FM, the combination of multimodal increase in pain sensitivity (Kosek et al., 1996) and dysfunction of descending pain inhibition (Kosek et al., 1997; Lannersten et al., 2010) suggests that the central nervous system plays an important role in the pathophysiology of FM. Specifically, studies have identified FM associated deficiency in activating e.g. rostral (r)ACC in response to pain, where rACC is believed to initiate the descending pain inhibition during painful stimulation (Jensen et al., 2009). Hence, a better understanding of the neurophysiological underpinnings of FM would be valuable for developing new treatments.

1.6.2.2 RA

Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease that primarily affects the joints. The prevalence of RA is estimated to be 0.5-1 % of the population of the industrialized world, and women and elderly are over-represented. The inflammation commonly leads to dysfunction and destruction of the bone in the joints, accompanied by severe joint pain.

However, the pain in RA is commonly accompanied by pain that is discordant with the degree of peripheral pathology (Thompson et al., 1997). Very little is known about the cerebral processes involved in pain processing in RA. In **study IV** we investigated resting state brain connectivity associated with prolonged pain in RA.

2 AIMS

The aim of this thesis was to investigate how intrinsic brain activity can be modulated by both pharmacological and non-pharmacological interventions, as well as by long-term exposure to chronic pain. Four specific aims were formulated:

- To investigate the influence of oral intake of two different and commonly used neuropharmacological drugs (benzodizepine and L-dopa) on intrinsic brain activity.

- To characterize the intrinsic brain activity in a cohort of fibromyalgia patients.

- To examine the effects of a physical exercise intervention in fibromyalgia on intrinsic connectivity and symptom improvements.

- To characterize the intrinsic brain activity pertaining to pain regions in rheumatism arthritis.

3 METHODOLOGICAL CONSIDERATIONS

In this section, I will briefly survey the methodological considerations pertaining to the studies presented in this thesis. For study specific methodological details on design, cohort demographics, data acquisition, preprocessing and data analyses, please see the relevant manuscripts in the appendix. For in depths discussions on the derivations, significance and meaning of resting state measures, see section 1.2.

3.1 COHORTS AND SAMPLE SIZES

For most of our studies (**study I, II** and **IV**), we used around twenty subjects per group. The proper sample size for imaging studies has recently been debated (Friston, 2012, Yarkoni 2009), were the converging conclusion is that the larger sample sizes the better, assumed that effect sizes are reported (Friston and Yarkoni, 2012). Although there exist software packages for conducting a priori power calculations of mass-univariate fMRI analysis (for instance the fMRIpower, described by Mumford, 2012), e.g. fMRIpower requires a priori ROIs specifications and estimations of effect sizes (which often is a complex function of first level design matrixes and the neural response). Instead, we relied upon the unofficial rule of thumb of around 20 subjects. This number probably originated from power calculations based on empirical and simulated data by Desmond et al. (2002), where they aimed for 80% power using uncorrected alpha value of 0.002 at a voxel level. Since our studies were somewhat pioneering and exploratory in nature, a priori estimate of power would have been very uncertain, why we stuck to these typical fMRI sample sizes. For **study IV** however, scanning sessions were discontinued for a high percentage of the healthy subjects, and many resting state scans (that were placed at the end of each run) were unfortunately lost.

For **study I** we tested healthy students in their early twenties. The subjects in **study II-IV** were in their late forties (in the FM cohorts) and early fifties (for the RA patients). In order to decrease any confounding effects of sex, only women were included in the FM studies (which reflects the fact that females are overrepresented in FM). In the RA cohort, a small fraction of the subjects were men, again mirroring the fact that RA prevalence is higher among women (although not to the same degree as in FM). By including patients using medications (except analgesics, NSAID, or hypnotics maximally 48 h prior to examinations), the number of available subjects increased and a more representative patient cohorts were obtained.

3.2 PHARMACOLOGICAL INTERVENTIONS – STUDY I

Subjects in the L-dopa group were orally administered Madopark, containing the active ingredients L-dopa (100 mg) and benserazide (25 mg, the latter is a decarboxylase blocker preventing peripheral side effects). This is a commonly administered dose of Madopark, aimed for Parkinson.

In the benzodiazepine arm of the study, subjects were given the anticonvulsant and anxiolytic medication Sobril (20 mg oxazepam), a dose that could be taken 3-4 times per day for more

severe anxiety.

3.3 PAIN ASSESSMENTS AND QUESTIONNAIRES – STUDY II-IV

For **study II-IV**, we used questionnaire to assess symptom gravity. These included the fibromyalgia impact questionnaire (FIQ) (Bennett, 2005), Short Form 36 (Contopoulos-Ioannidis et al., 2009) (of which only the body pain dimension, SF36BP, was used in the current studies). In **study IV**, severity of the rheumatic arthritis was quantified using the Disease Activity Score, DAS28, (Prevoo et al., 1995). Additionally, for the studies that characterized resting state correlates of rheumatic disease at baseline (**study II** and **IV**), the global pain perception was estimated using a visual analogue scale (VAS global pain). The VAS scale was a 100 mm long line on which the patients made a mark that corresponded to their overall pain intensity. Additionally, in **study II** and **IV** we further estimated pain sensitivity in response to experimentally induced pressure pain. The pressure pain stimulation was applied on the thumbnail (**study II** and **IV**) and on a rheumatic finger joint (**study IV**) by means of an automated pneumatic computer controlled stimulator that applied pressure via an 1 cm^2 rubber coated piston (Jensen et al., 2009). This enabled us to determine the amount of pressure applied to the subject's hands for which the subjects rated 50 mm on a 100 mm VAS scale. In the manuscripts, the resulting measure is referred to as P50.

3.4 RESTING STATE DATA PARADIGMS

Subsequent to task related fMRI scans (taxing decision making in **study I**, pain perception and conflict monitoring in **study II-III**, and only pain perception in **study IV**), 8 min and 20 seconds (200 volumes, TR=2.5s) of resting state data was acquired. During the resting state scans, subjects were told not thinking of anything in particular with their eyes directed at a fixation cross (**study I** and **IV**), or keeping their eyes closed **(study II-III**). One obvious advantage of using fixation cross is that it increases the chances that the subjects remain awake, and that it could enable the researcher to identify the extent to which the subjects fall asleep using eye-tracking (Fransson et al., 2014; Tagliazucchi et al., 2014). On the other hand, a conceivable advantage of using eyes-closed paradigm could be to allow more ecologically valid (or natural) spontaneous cognition, with a minimum of externally imposed task instructions.

3.5 RESTING STATE DATA ANALYSIS

In depth descriptions of the rs-fMRI measures are given in the section 1.2 above. For study specific application of the resting state measures, as well the precise choices of preprocessing parameters, please see the methods section of each study.

In large, preprocessing followed the conventional preprocessing pipeline as instantiated in SPM or FSL (MELODIC) (the latter in the case of the ICA reported in **study II)**. Thus, before inferential statistics, the EPI data was realigned in order to adjust for differences in head location across volumes within the same scanning session. To account for differences in slice time acquisition (that maximally differed by 2.5s, corresponding to the time to

repetition, TR), the volumes were slice time corrected (**study II** and **III**), although the costs and benefits of this preprocessing step is disputed (Sladky et al., 2011). In **study II-IV**, structural data was segmented into grey matter, white matter and CSF in order to improve structural normalization (**study II** and **III**). The segmentation output was later converted into binary tissue masks used to generate nuisance regressors pertaining to time series of white matter and CSF (**study II-IV**). In **study I** and **IV**, spatial normalization were performed directly (that is, EPI data were normalized using the EPI template supplied by the SPM). In **study II** and **III**, functional volumes were indirectly normalized by applying the same normalization parameters that were obtained from the structural normalization to the coregistered functional data. Finally, the functional data was spatially smoothed. The motivations behind spatial smoothing on fMRI data are firstly to improve the premises of normality underlying the statistical testing at the subject level. Secondly, smoothing increases the signal-to-noise ratio by increasing the probability of inter-individual overlap of brain activity, since spatial normalization unavoidably is imperfect and that there exists inter-individual variance in the functional anatomy. For analysis of regional homogeneity (**study I**), unsmoothed fMRI data was used.

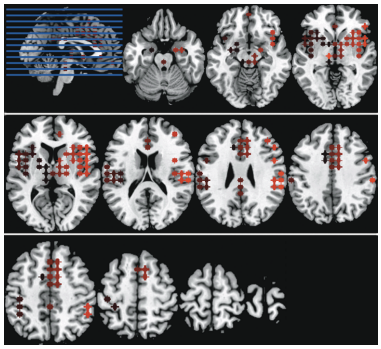

Figure 8. *Seeds located in brain regions involved in pain.* For SCA on rheumatic pain (study II-IV), we used 159 seed regions evenly spread out within pain processing brain areas as delineated by a meta-analysis on pain carried out in Neurosynth.org. The seeds are projected on an anatomical template brain provided in MNIcron.

In all SCA analyses, EPI data was filtered (0.1-0.01 Hz) to remove high frequency physiological (e.g. respiratory and cardiac) noise and low frequent noise due to scanner drift, in accord with conventional strategies. For the SCA analyses, first level nuisance regression typically included movement regression of the six rigid motion parameters retrieved from the realignment, as well as their temporal derivatives.

Additionally, we controlled for the time series of white matter, CSF (and in **study I** - the global brain signal), to obtain connectivity estimates that were as anchored in neuronal signal change as possible. Due to the recently raised awareness of the strong impact of movement on intrinsic connectivity (e.g. Power et al., 2012), rigorous measures to control for head movement has been taken at both the intra-individual and the inter-individual level. For instance, in the last three studies (**study II-IV**), the mean frame wise displacement (FD) was calculated and regressed out in the group comparisons, as advocated by Yan and colleagues (2013).

4 SUMMARIES OF STUDIES I-IV

4.1 STUDY I – NEUROPHARMACOLOGICAL MANIPULATIONS OF RS-FMRI

In this study we investigated the effects of neuropharmacological manipulations on resting state brain activity. One group of subjects (n=39) was randomly assigned to a double-blinded administration of either a single dose of L-dopa (100 mg) (n=19), and a control group (n= 20) that was administered placebo. In an independent cohort (n=42), one group (n=20) was given oxazepam (20 mg), and a control group (n=22) received placebo. For both group comparisons, we performed an identical set of analyses of resting state data. Firstly, we performed seed correlation analyses by defining seeds in brain areas with high densities of the cardinal target receptors of L-dopa (D1 and D2 receptors) and oxazepam ($GABA_A$ receptors). Secondly, exploratory analyses of group differences in fALFF and Cohe-ReHo were carried out. We hypothesized that oxazepam primarily would influence connectivity of the seeds located in regions with high $GABA_A$ receptor densities. Analogously, we expected a preferential modulation of connectivity of seed regions within brain areas rich in dopamine receptors.

These tentative hypotheses were not conclusively supported by the empirical results. Rather, oxazepam induced a general increase in connectivity, particularly of the DMN hubs in ventromedial prefrontal cortex and the PCC. Interestingly however, we observed an uncoupling of amygdala and temporal cortex, which speculatively could signify a potential anxiolytic mechanism of oxazepam.

On the other hand, L-dopa was primarily associated with a decreased connectivity, e.g. between the two cardinal nodes of the DMN, as well as between the amygdala and bilateral inferior frontal gyri. This study showed that single daily doses of commonly administered neuropharmaca indeed impact resting state connectivity. However, since the effects on the resting state measures were relatively subtle (compared to within group connectivity), a deeper understanding of the neurophysiology mediating the pharmacological effects would require increased statistical sensitivity. This could be attained by longitudinal (cross-over designs, see figure 9), larger sample sizes, or administration of parametrically increased doses. Furthermore, in order to understand the functional and ultimately the clinical significance of the drug induced connectivity modulations, apt behavioral and clinical measures need to be correlated with the rs-fMRI. Current results should guide formulation of hypotheses to be tested in future investigations of the mechanisms of L-dopa and oxazepam at the system level.

4.2 STUDY II – RS-FMRI CHARACTERISTIC OF FM

Although there exists putative peripheral factors involved in FM, there is still a perplexing dissociation between subjective pain and efferent noxious stimuli or peripheral injury. In addition to central sensitization, supraspinal reorganization is likely involved in the learning of pain (Jensen et al., 2009; Napadow et al., 2012). Since pain in FM is sustained (by definition chronic), rs-fMRI of the endogenous ongoing brain activity offers an attractive

complementary method to task evoked fMRI investigations of transient pain. Previous rs-fMRI studies in FM populations have reported FM associated increases in the connectivity between insula and the DMN (Napadow et al., 2012), disrupted sensorimotor coupling (Pujol et al., 2014), 2014) and a shift towards higher frequency content of spontaneous BOLD fluctuations (Kim et al., 2013).

In an attempt to replicate and expand on these findings, we scanned 17 FM subjects and 22 healthy controls using a typical resting state protocol. We then conducted a massive seed correlation analysis comprising 159 seed regions evenly distributed within cardinal brain regions, as demarked by an automated meta-analysis.

Contrary to our predictions, we could not replicate previously reported hyperconnectivity between insula and DMN. Likewise, no abnormalities of the frequency power distribution in DMN and insula were observed. However, the seed correlation analyses revealed FM associated weakened connectivity between multiple seeds regions located in pain areas and primarily sensorimotor brain regions. On the other hand, intraindividual differences in pain sensitivity correlated with increased connectivity between for instance posterior cingulate cortex and insula. Group differences in connectivity did not correlate with pain estimates. Thus, the functional significance of connectivity differences warrants further explorations. Speculatively, our findings could imply that abnormal connectivity patterns between pain-related regions and the remaining brain during rest reflect an impaired central mechanism of pain modulation in FM. Weaker coupling between pain regions and prefrontal- and sensorimotor areas might indicate a less efficient system level control of pain circuits. Moreover, our results show that multiple, complementary analytical approaches are valuable for a more comprehensive characterization of deviant resting-state activity. In conclusion, we have shown that FM primarily is associated with decreased connectivity, for example, between several pain-related areas and sensorimotor regions, which could reflect a deficiency in pain regulation.

4.3 STUDY III – EXERCISE INDUCED NORMALIZATION OF FM

In **study III** we investigated rs-fMRI brain connectivity before and after a 15 week standardized exercise program supervised by physical therapists. Our aim was to gain an understanding of how physical exercise influence previously shown (see **study II**) aberrant patterns of intrinsic brain activity in FM.

We studied the longitudinal effects of physical exercise in both FM and in healthy controls. We explored post- versus pre-treatment changes of brain connectivity, as well as changes in clinical symptoms in the patient group.

FM patients reported improvements in symptom severity as measured with the Fibromyalgia Impact Questionnaire (FIQ), but the body pain scores (measured with SF36BP) did not change. Although several brain regions showed a treatment-related change in connectivity, only the connectivity between the right anterior insula and the left primary sensorimotor area

was significantly more affected by the physical exercise among the FM patients compared to healthy controls.

These results suggest that the previously observed aberrant intrinsic brain connectivity patterns in FM become partly normalized by a physical exercise therapy. However, none of the observed normalizations in intrinsic brain connectivity were significantly correlated with symptom changes. Further studies conducted in larger cohorts are warranted to probe the precise relationship between improvements in fibromyalgia symptoms and changes in intrinsic brain activity.

4.4 STUDY IV – RS-FMRI CHARACTERISTICS OF RA

In **study IV**, we investigated if RA is associated with aberrant resting state functional connectivity in pain processing brain areas. For this, 24 RA subjects and 19 matched controls were compared with regard to both behavioral measures of pain perception and resting state fMRI connectivity as investigated using 159 seed regions located in cardinal pain processing brain regions. Additional multivariate pattern analysis was carried out in a data driven attempt to localize group differences in the patterns of whole brain functional connectivity.

When RA patients were compared to controls, we observed significantly lower pain resilience for pressure on the affected finger joints (i.e. P50-joint) and an overall heightened level of perceived global pain in RA patients. Relative to controls, RA patients displayed enhanced brain connectivity predominately for the supplementary motor areas, midcingulate cortex and the primary sensorimotor cortex. Additionally, we observed stronger connectivity between the insula and prefrontal cortex as well as within the default mode network for RA patients. None of the group differences in brain connectivity were significantly correlated with behavioral parameters.

Our study provides experimental evidence of aberrant brain connectivity in several pain related areas for RA patients compared to healthy controls. To better understand the clinical significance of this, future studies should relate rs-fMRI to disease severity in larger RA samples that for instance would allow for cohort stratifications.

5 GENERAL DISCUSSION

The aim of this section is to contextualize the main findings and assess the limitations of the studies. Furthermore, promising avenues for future research will be discussed, as well as developments of the rs-fMRI field at large. For in-depth discussion of the finding of each individual study, see the attached articles and manuscripts in the appendix.

An overarching aim of the thesis was to investigate the usefulness of rs-fMRI for characterizing clinical conditions such as rheumatic pain, and to study the malleability of intrinsic brain connectivity in response to single dose neuropharmacological interventions or a prolonged physical exercise intervention.

As evident from our four studies (as well as from the literature reviewed in section 1.5.2), intrinsic brain activity reflects neurophysiological process residing on different time scales. The findings from **Study II** and **IV** supports the notion that intrinsic brain activity can reflect exposure to long term disease, in accordance with a by now large number of other clinical rs-fMRI studies. The influence of long-term interventions on resting state connectivity was demonstrated in **study III**, and finally, plasticity on the timescales of minutes or hours were proven using pharmacological manipulations in **study I**. The functional and biological meaning of intrinsic brain activity at the various time scales likely differs accordingly.

In the studies presented in the thesis, we have showed group differences in intrinsic brain activity due chronic disease, administration of neurotrophic drugs and longitudinal changes following exercise. Although the group differences in brain activity are interesting per se, the largely lacking associating to behavioral parameters such as symptom gravity, limits the interpretations of their functional significance. Initial studies in any empirical research field are typically bound to be exploratory. As the science matures, more specific and crisp hypotheses can be formulated, allowing for stronger conclusions regarding underlying mechanisms of the object of investigations. To a large extent, **study I**, **II** and **IV** are initial exploratory studies, aimed at preparing the ground for subsequent, more model based research (Fox et al., 2010). Climbing the hierarchy of scientific evidence, succeeding the model-testing studies are intervention studies that allow for detection of mechanistic principles, that in the best of cases can shed light on causal relationships. **Study I** and **III** were both intervention studies, providing initial evidence of putative mechanistic factors. Increased inferential power is achieved by randomized, actively controlled trials, where the compared groups or conditions only differ with regard to the hypothesized "active ingredients", and all the other factors are ideally controlled for (see the limitations below). At the top of the hierarchy of medical scientific knowledge are systematic reviews and meta-analyses of randomized controlled trials. In the light of the accelerating rate of publications of rs-fMRI articles (as for medical publications in general), initiatives for integrating research findings and facilitating meta-analyses are laudable. Examples of neuroinformatics programs are the European Human Brain Project and the international neuroinformatics coordinating facility (INCF). For meta-analysis, applications like BrainMap gingerALE (Laird et al., 2011)

or web based Neurosynth (Yarkoni et al., 2011) allows for user-friendly data mining of a considerable and expanding portion of the (rs-f)MRI literature.

5.1 STUDY LIMITATIONS AND FUTURE DIRECTIONS

There are several aspects to be considered in future investigations that aim to extend on the studies presented in this thesis. Below I will address the most important ones.

5.1.1 Undisturbed rest

In all **studies (I-IV**), the resting state scans were acquired after task paradigms. As previously surveyed, many scientific papers report spillover effects of task unto rs-fMRI. There is a risk of interactions between group and the effect of scanning order. For instance, one could imagine that chronic pain patients experience stronger after-effects from a pain paradigm than healthy controls do, and that such asymmetry would confound group comparisons of the intrinsic brain activity. Thus, for this reason, the quality of resting state data would benefit from being placed in the beginning of the MR scanning session.

5.1.2 Increased statistical power

As already discussed, the perhaps largest drawback of many of the studies herein is the absent association between behavior and group differences (or in the case of study 3, between longitudinal improvement in pain symptoms and restoration of aberrant connectivity). Increased statistical power would most likely improve this issue. Increased power could be attained in three ways. Firstly, by increasing the sample size (N), the standard error of the mean decreases proportionally to $N^{-1/2}$. The parametric test statistic (e.g. the student t-statistic) thereby increases correspondingly. (See also further discussion on sample size and data sharing below). Secondly, increased power can be achieved by reducing the standard deviation and within group variance. Stratification of patient cohorts could be a means for obtaining more homogeneous groups. In the context of this thesis, given a for instance larger fibromyalgia cohort, dividing the group into subgroups based on symptoms (e.g. presence or absence of cognitive symptoms, sleep problems etc.) could be a promising approach. Similarly, controlling for confounding variables (such as head movement), and employing longitudinal rather than cross sectional designs (e.g. for the neuropharmacological interventions), would render a decreased unexplained variance. A third way to increase sensitivity is by increasing the difference between the null hypothesis and the true mean. In other words, increased sensitivity is obtained by increasing the effect size. One strategy for accomplishing this could be to enhance the experimental effects (perhaps using more efficient interventions or investigating patient cohorts with more severe pain symptoms). The second way would be to model the effect of interest in a more informed way. Thus, by making use of prior information, one could perform analyses based on more specific and elaborated psychological models. Statistical testing based on well-formulated psychological theories that make full use of the prior knowledge would increase the likelihood of exposing relevant

group differences compared to using more general, exploratory statistical models. Hence one would increase the chance of revealing larger effect sizes.

5.1.3 Refinement of behavioral assessments and physiological models

To facilitate the interpretation of reported group differences of intrinsic connectivity, clear associations to behavioral parameters would be needed. Thus, one valuable approach would be to identify relevant and reliable behavioral effects (of for instance the pharmachological or exercise interventions), and base the resting state analyses upon these (cf. the discussion at the end of the previous section). Any association between behavior and resting state activity critically relies on the validity, robustness and quality of the behavioral data. For example, one way of improving the pain estimates in the **studies II-IV** would be to acquire estimates of spontaneous ongoing pain or pain sensitivity in close proximity to the scanning session (as was done in Napadow et al., 2010). In general, relative to task-based fMRI, the link to behavior is typically more indirect in rs-fMRI due to the temporal distance between resting state acquisition and behavioral assessments. In task-based fMRI, the timing of the behavioral phenomenon of interest is typically well controlled and known, allowing for direct association between behavior and brain processes. The rs-fMRI scanning, on the other hand, is by definition uninterrupted for about 5-10 minutes. Quantification of any behavioral parameters to be correlated with the brain activity must thus occur before or after the resting state scans, unless the brain data and the behavior of interest can be recorded simultaneously (e.g. by using physiological recordings that do not disturb the resting state, as in Fransson et al., 2014). Development of dynamic resting state connectivity with higher temporal resolution (see section 1.2.3) in combination with intermittent though probes could in certain context decrease this temporal gap, and tighten up the connection between brain activity and behavior.

In the context of SCA, an improved selection procedure of seed locations, e.g. based on functional localizers, could facilitate interpretations of the functional connectivity. As a concrete example, in the RA study (**study IV**), a task based fMRI paradigm aimed at identifying the neuronal correlates of hyperalgesia during pressure stimuli over the most affected joints (not restricted to fingers) contrasted by the same stimuli over the corresponding but unaffected joint on the opposite side, could be used to obtain seed region of interests.

5.1.4 Improvement of the longitudinal rs-fMRI designs

One possible improvement of the experimental design in **study I** is to parametrically increase the amount of administrated drugs, for detection of potential dose-response relationships. Also, changes in medically relevant behavior could be assessed (e.g. self reported change in subjective experience). Additionally, implementing a double blind cross-over design would increase the statistical sensitivity by examining longitudinal, rather than cross sectional effects, since interindividual variance typically exceeds intraindividual variance (figure 9).

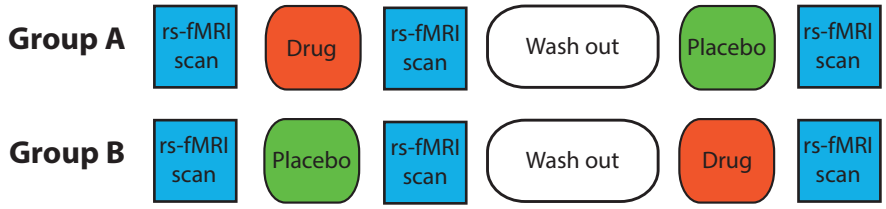

Figure 9. Example of a cross-over design in a pharmacological rs-fMRI study, which would enable within subject analysis while controlling for treatment order effects. A design similar to this has been utilized by e.g. Schaefer et al., (2014).

A possible improvement of the intervention in **study III** (in addition to increased sample size), would be to include an active control group. This would allow for investigation of the effect of the stipulated active mechanisms (i.e. the physical exercise) per se, while controlling for potential effects of regression towards the mean (Gibbons et al., 1987).

5.1.5 Promises of rs-fMRI and future outlook

Rs-fMRI research holds much promise for the future. I see several factors that likely will play central roles for advancing the rs-fMRI field: (i) Better control of physiological noise in the rs-fMRI signal (such as ICA based denoising techniques like FIX, see section 1.3), (ii) the development and use of more sensitive statistical methods (e.g. based on multivariate statistics and machine learning, that now are implemented in user friendly software such as the decoding toolbox, Hebart et al., 2015), (iii) inventions of reliable dynamic resting state measures that acknowledge the rich temporal information of rs-fMRI data, (iv) improvements in MR hardware and scanning sequences, and (v) conducting multimodal rs-fMRI studies by using complementing imaging technologies (DSI, ASL, MEG etc.). As the awareness of the replication crisis in medical science increases (Button et al., 2013; Schooler, 2014), one can expect improved research routines. Major grant agencies like the National Institute of Health (NIH) promotes sharing of both raw data and methodological tools, and calls for bigger study samples. Several large scale data sharing initiative have already been launched, offering high quality, freely available imaging data sets, often accompanied by behavioral data (e.g. the Human Connectome Project, Nathan Kline Institute -Rockland Sample, open fMRI, ADNI, and many more, see http://fcon_1000.projects.nitrc.org/). The need of larger sample sizes should foster more collaboration also between individual research labs. For instance, one could foresee increased data exchange between groups investigating similar subject cohorts, rendering multicenter studies with enhanced generalizability of research findings.

Recent initiatives on neuroinformatics (such as the INCF) facilitate access and extraction of general neurophysiological principles from large bodies of empirical data. There are also several newly launched pre-registered replication projects (like the Registered Replication Reports, http://www.psychologicalscience.org/index.php/replication), and an increasing number of important journals (e.g. the journal Perspectives on Psychological Science) encourage pre-registration of research programs in an attempt to tackle “HARKING” (“hypothesizing after the results are known”), which would hamper the file drawer effect. Underpowered studies often contribute to the positive publication bias in cognitive science (Ioannidis et al., 2014). Since only large effects survive significance thresholds in low

powered studies, in combination with a reluctance to publish null findings and a high pressure to publish, large effect sizes -spurious or true- will be overrepresented in the literature. In the former case, inflated effect sizes aka. “voodoo correlations”, will mislead the field (Vul et al., 2009; Yarkoni, 2009). By taking adequate measures, including pre-registrations of studies, increased publication rate of null findings, and using larger study samples, the publication bias could be curbed (Barch et al., 2013; Ioannidis et al., 2014). If not, research resources will be wasted on attempts to replicate and extend on scientific air castles, and the progression of science will suffer.

5.2 CONCLUSIONS

The work presented in this thesis report deviant intrinsic brains activity in rheumatic pain conditions. Moreover, we have studied neuronal plasticity in the functional connectome following pharmacological and physical exercise interventions.

In the rheumatic pain conditions such as fibromyalgia and rheumatoid arthritis, there are commonly discrepancies between subjectively reported pain and observable peripheral tissue damage. Thus, brain imaging of rheumatic pain could provide a means for bridging subjective pain and the related physiology. Biological markers of fibromyalgia would aid diagnosis and hopefully expand our understanding of the underlying etiology. In fibromyalgia we observed a pattern of decreased connectivity primarily between pain and sensorimotor regions. In contrast, RA was mainly associated with increased connectivity, such as between prefrontal midline regions and sensorimotor regions. In the absence of correlating behavior, the functional significance of these group differences remains speculative. One possibility is that RA patients have developed strategies of exerting frontal top-down control on somatosensory nociceptive input. FM, on the other hand, could be associated with an exhausted or otherwise decreased mutual regulation of brain regions coding for pain percepts and sensorimotor regions, thus perpetuating the pain symptoms.

We have showed effects of commonly ordinated single doses of dopaminergic and GABAergic drugs using rs-fMRI. These results should inform future attempts to reveal the mechanisms mediating pharmacological effects on the system level, by indicating plausible effect sizes and demarking the implicated connectivity. Likewise, we have observed support for the beneficial effects of physical exercise for fibromyalgia patients. These likely involve restorations of connectivity between insula and sensorimotor regions. Possibly, this could reflect a more coherently working system level control of pain processing. The ‘‘interoceptive thermostat’’ represented in the anterior insula, displayed stronger functional connectivity to areas controlling for locomotion and body representations, following the exercise intervention.

Intrinsic brain activity reflects aspects of brain organization that have relevance to personal traits, disease and ongoing behavior. The precise biological and cognitive meaning of rs-fMRI measures is an important topic for future research. To enable applications of rs-fMRI

findings, high scientific reproducibility is required. The many promising international initiatives of data sharing reflect this need. Once the initial and explorative studies has laid a ground for more model based research, rs-fMRI studies that draws maximally on prior research findings will accelerate the advancement of the field. Considered the importance of neuroscience in health care and in the society at large, the potential impact of rs-fMRI is grand.

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