When default is not default: Solutions to the replication crisis and beyond

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Summary: This project addresses a very timely and highly important question of cognitive neuroscience studies: What factors influence the reliability of neuroimaging studies? Recent studies have estimated the reproducibility of psychological studies to be 39% or less and indicated a severe limitation of neuroimaging (fMRI) study reliability. Too small sample sizes, low to moderate effect sizes, and only partly understood neurophysiological mechanisms behind the BOLD/fMRI signal make it difficult to generalize results, thereby impeding the impact of highly needed neuroscience studies on theoretical (scientific), methodological, and clinical progress. The overall objectives of this project are to (i) improve our understanding of the neurophysiological mechanism of the BOLD signal and its sources of variability to (ii) find a solution to the replication crisis by developing a new Bayesian and topology-based resting-state analysis framework as an alternative approach to today's analysis strategies, and to (iii) induce a paradigm shift from the current focus on an easy to measure but susceptible BOLD signal to the underlying, but (partly) hidden neuronal states that are presumably more stable and reliable. The project will break new ground by providing new insights into the neurophysiological mechanisms of the BOLD signal, its variability, dependency on endogenous and exogenous parameter, and reliability, and it will advance the research field of basic and clinical neuroimaging by providing new analysis strategies. To accomplish its mission, this project will use a longitudinal approach to examine the variability and reliability of resting-state networks and brain dynamics, supplemented by a cohort study in healthy participants as well as an exemplary clinical study and by the analysis of fMRI data from open-access data repositories. The PI, Prof. Karsten Specht, is a highly experienced researcher in the field of functional neuroimaging with expertise in fMRI and methods development. Together with renowned and leading international experts in the fields of neuroimaging and methods development, he will (if awarded) use this project grant to build upon the excellent research environment at the University of Bergen and extend it into an internationally competitive research group on applied neuroimaging. The proposed novel analysis framework and the aim to induce a paradigm shift at the intersection of basic and clinical research will allow for the generation of yet unthinkable research questions, and thereby put the PI into an optimal position for a future ERC Advanced Grant.

1 Aspects relating to the research project

1.1 Introduction

1.1.1 The replication crisis and its consequences

As recently recognized, the entire field of psychology suffers from the "replication crisis" [1-5] with a reproducibility of psychological studies of about 39% or less. And also for neuroimaging studies the reliability of the results is limited[6]. The research community has taken this very seriously and the Organisation of Human Brain Mapping (OHBM) announced in 2016 a new replication award, and put reproducibility high up on their agenda with several new best practice and data sharing initiatives (see e.g. http://www.ohbmbrainmappingblog.com).

Jointly, psychology and neuroimaging suffer substantially from a lack of statistical power, meaning that the sample sizes are typically too small and effect sizes are only low to moderate, making it difficult to generalize study results to larger populations. This has not only been perceived as a critical challenge among scientists, but has recently also received public attention. Although the limited reliability is known, neuroimaging is used in an increasing number of clinical studies, where not only group but also single-subject assessments are conducted. However, knowing about the intra- and inter-individual variability, only group studies are currently justifiable, where this variability is averaged out – at least if samples sizes are large enough. Consequently, all clinical single subject approaches are highly questionable.

The recent years have seen a tremendously increased interest in resting-state or intrinsic-brain networks and brain dynamics[7-9]. These are networks that can be identified with resting-state fMRI (rs-fMRI), where no explicit task is given to the examined person. It has been shown that even in the absence of a concrete task, certain brain areas show characteristic network patterns, called resting-state or *intrinsic network*. These network patterns can be detected by focusing on low frequent (<0.01Hz) fluctuations of the BOLD signal, since these fluctuations propagate through the underlying neuronal network structures, indicating an information exchange within the networks even in the absence of a concrete task. It has been further shown that these networks are very similar across individuals. They are therefore assumed to reflect some fundamental – trait-like – brain processes. Some of these networks have been identified as intrinsic brain networks that occur in the majority of healthy subjects although with some systematic variability[10,11].

One of the most investigated networks in this respect is the "Default Mode Network" (DMN)[9,12]. The DMN network is related to processes like mind wandering, intrinsically focused attention, daydreaming, etc. Other resting-state networks are associated with sensory processes, extrinsic attention, or language, to name a few. Interestingly, the counterpart to the DMN is the "Extrinsic Mode Network" (EMN), which represents a network for extrinsically focused attention, and current research focuses on the interplay between them[9].

The simplicity of this technique and the absence of a concrete task makes rs-fMRI to an ideal tool for clinical studies, especially to those studies where patients are severely affected like after a stroke or traumatic brain injury, but various other neurological and psychiatric disorders, like Parkinson's Disease, depression or schizophrenia. Clinical applications of rs-fMRI rest on the assumption of certain inter- and intra-individual stability of resting-state networks in healthy individuals in order to draw conclusions from observed deviations in patients. In order to increase comparability and to limit variability in data acquisition between studies, a first set of guidelines for standardized protocols has been developed (e.g., like the "Alzheimer's Disease Neuroimaging Initiative" (ADNI); http://adni.loni.usc.edu), and other initiatives are following their example and have started similar undertakings.

However, the strength of rs-fMRI is also its weakness. The absence of a concrete task for the participant during the investigation potentially increases the problem of limited reliability, since the nature of this study-technique incorporates the problem that one cannot control what the person in the MR scanner is actually doing while being examined. But, resting-state fMRI studies are based on the assumption of an inherent stability of the underlying resting-state networks across time and individuals. In other words, they assume a low intra- and inter-subject variability with high sensitivity to clinical deviations. This assumption has, however, never been thoroughly tested and might not be justifiable. There are only sparse and inconsistent reports that resting-states are indeed resting-traits[13], while the majority of reports point out that intra-individual variation can depend on, e.g., the time of the day, time of the year, prior events, or mood[14-16], and concerns about other endogenous and exogenous sources of variance have been raised during recent years[17].

One of the major knowledge gaps in the field is the assumption that the fMRI signal, i.e. the underlying BOLD effect (BOLD=blood oxygenation level dependent), is *sufficiently* reliable, where "sufficiently" has never been defined yet. It is of crucial importance to keep in mind that the BOLD signal represents only an indirect measure of neuronal activity, through a cascade of physiological processes (see, e.g. Balloon model in section 1.1.3). Consequently, the observed variability of the BOLD signal does not necessarily justify the conclusion that also the underlying neuronal activity shows variability to the same degree. Scientifically speaking, the BOLD signal is a physiological response that only indirectly reflects neuronal activity, and which is easily and directly influenced by blood pressure, blood oxygenation, or any other parameter that has an effect on the vascular system, which in turn influences the Balloon effect that generates the BOLD signal[18,19]. However, the neuronal activity and the connectivity within the neuronal network might be unchanged. The challenge and the aim of this project is to identify endogenous and exogenous sources of variability, and to develop data acquisition methods as well as data analysis methods that allow to reliably inferring on the unobservable *true* neuronal states given the measured BOLD signal, since only the latter is assessable through fMRI. In order to achieve that goal, this project will, for the first time and together with leading experts in the field, combine exhaustive data collection from each participating individual with up-to-date data analysis, modelling, and simulations techniques, including deep learning, and new analysis strategies that are less susceptible to BOLD signal variations that are not causally related to neuronal activity.

The project will, for the first time, provide a justifiable basis for all ongoing and planned rs-fMRI studies, especially for those, which aim to compare resting-state networks between healthy participants and patients, suffering fro neurological and psychiatric disorders. Those studies will only be successful when the variability within the population is known and possible sources of variation are identified, measureable, explained, and regressed out. Based on those estimates, researchers will be able to perform more accurate power analyses for calculating realistic sample-sizes for clinical and comparative fMRI studies. The outcomes and deliveries of this project will substantially improve reliability of rs-fMRI, it will provide new and improved analysis tools, it will present precursors for new clinical analysis tools for rs-fMRI and task-related fMRI, but will also be applicable for other approaches, like (resting-state) EEG/MEG.

Most importantly, the outcomes of this project will result in a better understanding and an improvement of the widely used neurophysiological model that describes how neuronal activity maps onto the measured BOLD signal (a brief description of the Balloon model is given in section 1.1.3 and Figure 1)

1.1.2 The current approaches in resting-state fMRI and their limitations in clinical studies

The current approach in rs-fMRI is to conduct an fMRI scan of 5-10 min duration with either eyes closed, eyes open, or eyes fixed on a fixation cross. Although differences between these three possible instructions are moderate, they are still measurable[20]. It is, however, difficult to control how well an individual follows that instruction as eye-tracking devices or eye cameras are typically not installed inside of an MR scanner and especially not in clinical MR scanners. Although differences might only be moderate, in the light of clinical applications, they may be in the same range that differentiates between patients and healthy controls.

Another source of variability is the time of the day and time of the year during which the measurements are done. In an effortful longitudinal study of a single subject over 3.5 years, Choe et al. could show that there were systematic variations with a "significant linear trend, annual periodicity, and persistence"[21]. Others have found that resting-state activity varies with the circadian rhythm[22], prior events[14], or mood[16]. Another factor that varies between different studies and also influences the results is the duration of the resting-state examination that roughly varies between a few minutes and up to 10 minutes and more. Reliability of specific rs-fMRI measures appear to scale almost linearly with the duration of the measurement[21].

Previous studies have applied a wide spectrum of rs-fMRI analysis strategies, with varying levels of reliability[23]. One of the standard methods is the application of independent component analyses (ICA) that splits the data into separate, independent networks. The advantage of ICA is that it does not require anatomical a-priori hypotheses where activity or differences are expected to occur as well as it allows to infer on underlying network structures. On the other hand, ICAs have repeatedly been criticised because it is not well defined how many ICA components one should expect or extract from the data. The literature broadly varies between 20 and 100+ components. The problem is that individual or pathological variability might be overlooked when the number of extracted components is too low or too high, since they easily can disappear in either the estimated uncertainty of a component or in one of the hundred (unexplored) components. Clear standards are missing and the algorithms that approximate the number of expectable components (like the Akaike Information Criterion (AIC)) are often thought to underestimate the number of components. Hence, studies that use different numbers of components are less comparable with each other. Further, ICA-based methods are limited to clinical applications of rs-fMRI as patients may present normal resting-state networks with low numbers of extracted components and may appear pathologic with higher numbers.

Another often used method for rs-fMRI is seed-based analyses. In contrast to ICA, seed-based methods are dependent on a-priori anatomical hypotheses as the underlying principle is the correlation of the time-course of one pre-defined brain area with all other brain areas. Consequently, the method depends on the selection of the "seed voxel". This method is relatively popular for the examination of the DMN, by using the posterior cingulate cortex (PCC) or precuneus as seed regions, which then typically shows correlated activity in the parietal lobe as well as ventral anterior cingulate cortex and orbito-frontal areas. The advantage of that method in the clinical context is a better comparability between populations, since comparisons can be based on the correlation estimates. On the other hand, one is limited to the a-priori defined network, like the DMN. Differences in other networks might be overlooked or they have to be tested with different seed regions – which in turn raises a multiple-comparison problem when too many seed regions/networks are used. Between-study variability already arises in the way how confounding noise components are treated, like technical noise from the scanner, movement, physiological noise, etc. that typically propagate as highly correlated information through these analyses and may result in biased correlations. To circumvent this problem, some research groups regress out the signal from white matter and CSF, but problems – again – arise from where these noise components are extracted and whether one should include a "global signal" as well. This is currently a hotly debated issue among researchers.

Besides those purely seed-based analyses of time courses, various other methods focusing on the characteristics of the time courses have become increasingly popular. One approach is the analysis of regional homogeneity[24] that estimates the similarity of time series in neighboured voxel. Other methods analyse time spectra and spectral density of BOLD signal fluctuations and infer from there on deviations in activations between populations, like detecting shifts to higher or lower frequencies or changed dispersions.

That information can be, for example, extracted from ICA analyses and the component-related time courses. Multivariate methods have also been introduced, like multivariate pattern analyses that were originally developed for task-related fMRI but got extended into the field resting-sate fMRI, as well.

All introduced (and also other here not introduced) methods have been applied to various clinical studies in depression, schizophrenia, pain, stroke, traumatic brain injuries, developmental disorders, neurological degenerative disorders, etc. But it has been repeatedly questioned how informative and how sensitive these

procedures are, since different methods may vary in their specific conclusions that can be drawn, although overall pattern might be comparable[25].

1.1.3 Balloon model

The Balloon model became the most influential and mostly used model in fMRI research[18,26,27]. It is a neurophysiological model that describes the neuronal and vascular mechanisms that cause the BOLD signal given a neuronal activity (see also Figure 1). It rests on the assumption that the BOLD signal is caused by changes in the blood volume, blood flow and the oxygen extraction rate. It is widely accepted that these are the main parameter that determine the strength of the BOLD signal. The Balloon model is, for example, an integral part of several analysis models of fMRI data, like dynamic causal modelling (DCM). Simplified, DCM allows inferring on hidden states through generative models and model inversions: First, hypothetical neuronal activations (e.g. neuronal-mass models) are used for generating hypothetical BOLD signals by applying the Balloon model. Second, hypothetical and measured BOLD signal are compared and, through model inversion, the initial hypothesis on neuronal activation is modified. This iterative process continues until it converges. Needless to mention, this procedure depends on a correctly specified Balloon model.

However, it is less studied, how susceptible the BOLD signal is to changes and individual variability of the underlying mechanisms. The core question is: Do parameters that influence the blood volume, blood flow, oxygen extraction rate, or neuronal activity per se have a substantial – and non-negligible – influence on the BOLD signal? For example, it is known that cortisol, blood pressures, or body-mass index (BMI) have an influence on blood volume and blood flow [28-30]. Whether individual variability of these parameters has a significant influence on the BOLD signal is largely unknown. To give another example: Using magnetic resonance spectroscopy (MRS), it has been shown that the individually varying concentration of the inhibitory neurotransmitter GABA is reflected in the amplitude and shape of the BOLD signal [31]. Complementary, we have shown comparable effects for the excitatory neurotransmitter glutamate [32,33]. The list of those endogenous parameters can be continued, including parameter that may predominantly affect neuronal signal transmission or vascular processes.

1.2 Overall research objectives

This project asks two simple but important questions: First, what is the inter- and intra-subject variability of the resting-state networks, measured through the BOLD signal, and which factors may influence this? Second, is the underlying neuronal network and network activity less susceptible to these factors?

To answer these questions, this project follows an integrated four-folded strategy: 1) analysing data from open-access repositories for estimating power, effect size, and variability in large samples; 2) acquisition of longitudinal and cohort data, including various endogenous and exogenous parameter; 3) neuroinformatic approach with simulating and modelling of data; 4) development of new analysis strategies, including new analytic and deep-learning approaches. The ultimate test of the outcomes and deliveries of this project will be the application to rs-fMRI data from patients with neurological and psychiatric disorders (see also Figure 2 in section 2).

In the initial phase of the project, data from open-access repositories will be used for estimating power and required sample-sizes for both a longitudinal and a cohort rs-fMRI study. Main effort of the project will be to explain and regress out the observed variability of these two rs-fMRI studies for increasing reliability of resting-state network and brain-dynamic estimates. This will be done using available software solution for analysing, modelling and simulating rs-fMRI data (dynamic causal modelling (DCM), the virtual brain (TVB), independent component analysis (ICA)). This will be supplemented and improved by developing new multivariate and neurocomputation approaches that will combine rs-fMRI (acquired longitudinally from the same persons on different MR scanners) with several other endogenous and exogenous measures that are typically not collected in regular rs-fMRI studies, but have been shown to influence resting-state activity[14-16,21-23]. These measures will comprise, for example, levels of cortisol and sex hormones, regional concentration of neurotransmitter (glutamate, GABA), regional brain perfusion, blood pressure, heart-rate variability, BMI, time of the year and time of the day, as well as resting M(E)EG-activity. It is expected that the (joint) influence of these additionally acquired parameter on the BOLD signal can be inferred by examining the naturally occurring variability within and between individuals. It is aimed and expected that this project will explain a substantial amount of the observed variability in the BOLD signal. Further, it is expected to show that this observed variability does not reflect a variability of the underlying and hidden neuronal activation and network structures but a variability of the vascular coupling mechanisms. Besides the acquisition of real in-vivo data, neurocomputational modelling and simulations will be performed in order to determine and model stability and variability across rs-fMRI in general, but also across different methods for analysing rs-fMRI data. Finally, a new multivariate and multimodal, Bayesian, and topology-based resting-

state analysis framework will be put forward as an alternative approach to today's analysis strategies. This new framework will focus on inferring on the hidden neuronal states and network dynamics (e.g. DMN vs. EMN), which are expected to be more reliable than the BOLD signal itself.

1.3 Significance of project outcomes and deliveries

1.3.1 Outcomes

The outcome of this project will not be less than revising and improving one of the most fundamental models used in functional neuroimaging. A better understanding of underlying physiological mechanisms will lead to an improved implementation of this model, which will lead to improved inferences on hidden states, since more variability of the measured BOLD signal can be explained. Consequently, this will improve reliability; not only for rs-fMRI but also for fMRI in general, including prospective clinical applications.

Further, a better knowledge of sources of variability will allow a better estimation of power and sample sizes, since todays low reliability might be a consequence of underpowered studies. Therefore, this project will deliver important guidelines on how sample sizes and power should be estimated.

1.3.2 Deliveries

New analysis strategies will advance the way how neuronal states can be inferred from the measured fMRI signals. By combining simulations, modelling, multimodal integration, topographic analysis, and deep-learning strategies, this project will develop new algorithms and analysis tools. They might be used as stand-alone applications or might be incorporated as extended modules into existing solutions, like the most widely used software SPM (Statistical Parametric Mapping) and GIFT (Group Independent component analysis of fMRI Toolbox), where the respective PIs of both software solutions are project partner.

2 The project plan, project management, organisation and cooperation

2.1 Solving the replication crisis

The current state-of-the-art in the field of clinical neuroimaging is based on large (multi-centre) studies focusing only on well-selected and predefined patient cohorts that have received a clear diagnosis based on standard clinical assessments, that vary in their study design, and which apply different (often not comparable) analysis methods. The proposed project will go beyond this by assessing parameters that are in principle observable but typically not included in clinical and basic-research fMRI studies, irrespective whether these are task-related or rs-fMRI studies. If fMRI, and particularly rs-fMRI, should enter a more clinical perspective, one has to develop methods that are sensitive to the small deviations, while regressing out known confounds. Clinical fMRI needs to be sensitive to small deviations in general, and especially to deviations in an early stage of a disease. This is only possible when typical sources of variability are identified and their influence is quantifiable.

The current formulation of the Balloon model, which is one of the mostly used models for explaining the origin of the BOLD signal, does not take into account parameters that may affect the vascular coupling. Hence, observed inter- and intra-subject variability is difficult to explain and may be misinterpreted as variability on the neuronal level, while it might be only variability of physiological mechanisms. To overcome this, the proposed project will collected a bunch of additional measures that are typically not examined, although they are easily accessible and are known to have an influence on neuronal activity or on the vascular system. It is expected that taking into account these additional measures and incorporating them into the Balloon model, a more reliable estimate of neuronal activity will be possible. This will further improve our knowledge on the neurophysiological mechanisms behind the BOLD signal and will advance our way of analysing and modelling fMRI data.

2.1.1 Overall hypothesis

Figure 1 explains the theoretical concept of the proposed project: The observed BOLD signal is the end product of a cascade of different mechanisms. These mechanisms are triggered by neuronal and synaptic activity, causing also an activity of astrocytes, like the glutamate-glutamine cycle, which is one of the main contributors to the blood flow increase. However, these processes are not directly observable through fMRI studies and could only be inferred, given the BOLD signal. In order to capture more information about these hidden neuronal processes, electrophysiology (M(E)EG) and concentrations of neurotransmitters will be collected. Further, sex hormones are discussed to have an influence on the excitatory and inhibitory balance, especially in females throughout their menstrual cycle, but also naturally occurring fluctuations of hormones might show an influence, as well. As outline before, neuronal activity causes changes in blood flow, blood volume, and oxygen extraction rate. However, these processes might be influenced through parameters that are known to affect the vascular system, like blood pressure, BMI, hear-rate, consumption of coffee and

nicotine, and, indirectly, through cortisol. By today, it is largely unknown, to which extend the mentioned parameter affect the reliability and account for the inter- and intra-individual variability of fMRI studies.

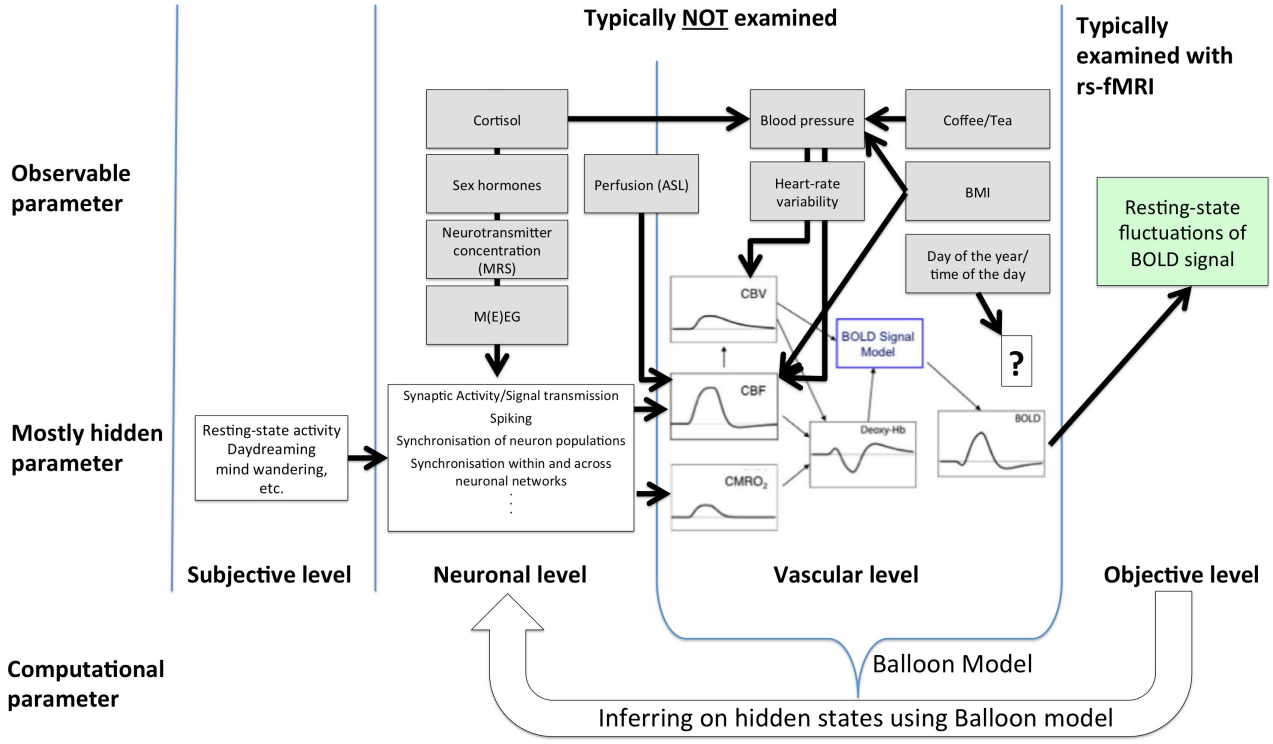

Figure 1: The different levels of explanations/working hypothesis: The BOLD signal is the measurable endpoint of a cascade of unobservable different vascular and neuronal mechanisms that is used for inferring on cognitive states. Up to now, resting-state fMRI examines only the fluctuations of the BOLD signal (green box), without controlling for parameters that may – or are known to - influence the processes on the neuronal and vascular level (grey boxes). Consequently, inference on neuronal and cognitive states can be improved and may become more reliable when the parameters are taken into account. All four subprojects aim to examine and explain the observed and mostly hidden parameters for inferring on the neuronal level. In addition, subproject 3(SP3) will focus on the computational part by developing new analysis strategies (Note: Figure describes an extension of Buxton's Balloon model[18]).

2.1.2 Project structure

The overall objective of this project is to examine inter- and intra-individual variability in fMRI studies, in particular resting-state fMRI, to identify possible exogenous and endogenous sources of variability, and to develop new analysis strategies that identify more reliably the underlying neuronal sources rather than being susceptible to the variability of the fMRI signal. To address this issue, the project will be divided into four main subprojects and a fifth clinical subproject (see Figure 2): SP1: Open-access data; SP2: a longitudinal rs-fMRI study, SP3: Neuroinformatics, SP4: a cohort study. In addition, SP5 will conclude the project by applying the new methods and outcomes to an exemplary clinical study.

The data from the repositories will be used to extract information about inter-individual variability and possible age-related effects. Further, these data will be used for estimating the power and final sample sizes for the subsequent longitudinal and cohort study. The data from the longitudinal and cohort study will be used not only for confirming the measures from the repositories, but also to examine inter-/intra-individual variability, and to control for other exogenous and endogenous factors, that are undocumented in the data from the repositories. These are, for example, time of the year, daytime, hormonal status, scanner stability measures, regional concentrations of neurotransmitter, resting-state EEG etc. Using these three primary data sources as starting point, data will be analysed with both existing software as well as to be developed software that rest on Bayesian statistics, deep-learning, multivariate as well as topographic data analysis strategies. Of particular interest is hereby the analysis of network interactions of the EMN and its counterpart, the DMN. Further, neurocomputations and -simulations will be added to examine and extrapolate the discovered effects. Finally, the deliveries and outcomes of this project will be exemplarily applied to existing and to be acquired data from patients, suffering from Parkinson's Diseases. This patient group has been selected, since this neurological disorder is dominated by disturbed neuronal activation and synchronisation, and there is increasing evidence of deviating rs-fMRI activity (publication in preparation) – but only on a group level; individual variability and symptom related rs-fMRI assessments are understudied.

2.2 Method

2.2.1 Participants

The study group of this project will be a sample of in total 180 participants (90 males, 90 females) with an age between 20 and 30, mixed education, representing the general population as close as possible. About 60 participants will become part of the longitudinal study with in total 8 multimodal examinations, and the remaining participants will form the cohort group. Participants will be characterised in terms of education, handedness, age, gender, mood, and, at each occasion, hormonal status through saliva samples (cortisol, testosterone, progesterone, and estradiol), blood pressure, heart-rate variability (HRV), body-mass index (BMI), as well as current alcohol, nicotine, tea, and caffeine consumptions. These parameters are known to affect the BOLD signal and/or neuronal firing, but they are typically not collected or documented and their effect on the reliability is unknown. Neuropsychological testing will give a profile of important cognitive functions, like working memory and attention (see table 1). Exclusion criteria for all participants are neurological or psychiatric disorders, implants, claustrophobia, large tattoos, and pregnancy.

2.2.2 Data acquisition

2.2.2.1 Open-access neuroimaging database

Data sharing is of increasing importance in the field of neuroimaging, as also emphasized by one of the project partners, Vince Calhoun[34]. The Human connectom project (http://www.humanconnectome.org), to name one of several databases, includes an open-access neuroimaging database of about 900 MRI datasets. In the initial phase, subproject SP1 will examine the inter-individual variability that is present in the data of the database. This will secure an efficient use of resources since this approach allows the estimation of the power, effect and sample sizes that can be expected in the longitudinal and cohort study (SP2 & SP4). The data from the repositories will also be used to examine and to compare the inter-individual variability that typically occurs in these samples, and the data from these repositories do also allow an extrapolation of these measures to include aging effects, which is a factor that might be considered later as this project progresses.

2.2.2.2 Longitudinal rs-fMRI

The central part of this project is a longitudinal examination of a well-characterised cohort of 60 participants, aged 20-30 (30 males / 30 females). The proposed number of participants should be interpreted as a rough starting point, as it might change once power and sample size have been estimated from the open-access data (SP1). Participants will be scanned 8 times during the project period (see Table 1), 4 times on the GE-750 MR system, 4 times on the Siemens PRISMA MR system, at varying times of the day and year (in particular dark and light periods of the year), in addition to EEG (and, if available, MEG) measures. The MRI scanning will follow the ADNI 2 protocol with structural imaging, diffusion tensor imaging (DTI), rs-fMRI (eyes open, eyes closed, fixation), perfusion measurement with arterial spin labelling (ASL), as well as MR spectroscopy (concentration of Choline (Cho), Creatine (Cr), Glutamate/Glutamine (Glx), myo-Inositol (mI), N-Acetyl-Aspertat (NAA), and GABA) from posterior (DMN) and anterior cingulate cortex (EMN). At each time point, saliva samples will be collected before and after the examination, and results will be averaged.

2.2.2.3 Cohort rs-fMRI

Data from the longitudinal study will be supplemented with one-time scanning of 120 additional participants from the same population (60 males / 60 females) randomly assigned to one of the MR scanners and spread over the entire project period to cover all times of the year. The same parameters as mentioned above will be collected. Note: To reduce variability, the current project will limit the factor “age” to the range 20-30 years.

2.2.2.4 Concurrent EEG and fMRI

Following our earlier studies [35-38], the study protocol will include concurrent electroencephalography (EEG) and fMRI examinations. The advantage of including EEG is that it will provide additional information about brain dynamics, neuronal activity and synchronisation. In particular the synchronisation of neuronal activity is reflected by synchronisation and increased power and coherence in different frequency bands. The underlying brain functions can be inferred from the frequency band in which the synchronisation occurs [39-42]. If it becomes available during the project period, these measurements will be supplemented by magneto-encephalography (MEG) examinations that yield even more precise results when coupling with EEG. MEG further improves the localization of signal sources and the study of fluctuations in brain dynamics.

2.2.2.5 Neuropsychological testing

All participants will be examined with a set of standard neuropsychological tests to characterize their capacity for executive functioning, working memory, and emotional state. The tests will include dichotic listening with attentional modulation, the California Verbal Learning Test (CVLT), Stroop test, and a mood

questionnaire. These tests will be performed prior to study inclusion (T0), and, for the longitudinal group, during the study (T4) as well as at the end of the study period (T8). This will reveal how stable these measures are over a period of three years, by limiting learning effects (only one test per year).

2.2.3 Data analysis with existing tools

In recent years, several advanced neuroimaging and neurocomputing tools haven been developed. Among these initiatives are applications like the human connectom project and related to it "The virtual brain" (TVB) as well as dynamic causal modelling (DCM) that explore the functional and effective connectivity in human brain networks. All electrophysiological data will be analysed using standard software, in addition to EEGLAB and SPM software as well as standardized low-resolution brain electromagnetic tomography (sLORETA). The fMRI, structural, ASL, and DTI data will be analysed with standard analysis tools, using both univariate analysis methods, like the general linear model as implemented in the software package "Statistical Parametric Mapping" (SPM), and multivariate methods, like independent component analysis (ICA)[43]. The latter one can also be used for multimodal integration for combining structural and functional imaging, but also EEG and MEG. MRS data will be analysed with LC-Model. Saliva samples will be analysed in the biochemical lab at the Department of Biological and Medical Psychology.

2.2.3.1 Dynamic Causal Modelling (DCM)

DCM, developed by the project partner Karl Friston, uses generative models and Bayesian Statistics to examine the functional – i.e. static – as well as effective – i.e. context dependent – connectivity. In order to conduct a DCM analysis, a model on network connectivity is defined, which is used for formulating a generative model. This model rests on the earlier described Balloon model, and is used to predict a possible fMRI signal. Through the iterative inversion of the model and adjusting of the initial parameter of the generative model, DCM is able to estimate the possible causes of the observed effects[44]. Hence, a DCM model gives a hint, which neuronal processes might have caused the observed data, since the true neuronal activations are not observable with in-vivo fMRI. Further, the DCM framework can be applied to task-related fMRI, rs-fMRI as well as EEG and MEG. The newest release of DCM includes also model estimates that rest on cross-spectral density[27], which are mostly relevant for rs-fMRI.

Estimated DCM parameters do not only include information about connectivity pattern, which are typically reported in DCM studies, but they also include parameters that are related to the Balloon model (see, for example, our recent publication[45]). These are the parameters that are of special interest for the present project, as they also allow the prediction of local neurotransmitter concentrations and hormones (data from an own study, publication in preparation).

Generally, DCM makes use of the Bayesian framework with Bayesian model selection and comparison as well as parametric empirical Bayesian inferences as core methods for the determination of the most realistic and explanatory model. For the present project, this is going to be one of the core methods as the underlying, hidden causes are expected to be more reliable than the fMRI signal itself.

2.2.3.2 The virtual brain (TVB)

Unlike the DCM approach, the TVB design rests on the incorporation of functional and structural connectivity data (rs-fMRI, DTI) into the estimation of Neuronal Mass models, which in turn can be used to simulate neuronal connectivity and activity patterns that have the potential to influence functional whole brain measurements[46-48]. This approach, developed by project partner Viktor Jirsa, is directly linked to the "human connectom" project (http://www.humanconnectome.org) and is applicable to rs-fMRI, EEG, and MEG. Recent applications of TVB have demonstrated that this tool can even be used in clinical research to explore a disorder (e.g., epilepsy) and to examine how neuronal activations and connections may have to change in order to generate a more normal activation pattern – some call this approach "virtual therapy". In the same way, causes and consequences of individual variability can be incorporated in TVB models, given the measured variability from the longitudinal as well as the cohort study. This will make it possible to extract further parameters that potentially can influence reliability measures of fMRI, i.e. reliability measures that only focus on the BOLD signal without controlling for possible underlying mechanisms.

The combination of TVB and DCM models within the same project is a new approach that has never been done before. This combination will elevate the sensitivity in studying intra- and inter-individual variability and reliability. The generated models allow inferring on possible deviations and temporal variations in brain networks *("Conenctoms"*). Further, it can be examined how intra- and inter-individual variability, exogenous and endogenous factors, as well as any kind of disturbances of the network may explain the observed variations. This has several substantial advantages. For the present project, the last rs-fMRI examination of the longitudinal sample will be used as confirmation for the TVB-based simulations, once sources of

variability have been identified, but also for the topologic data analysis and deep-learning analysis. Analyses should confirm improved reliability and successful identification of sources of individual variability.

The findings of this project can be used in future projects: The models can be used for simulating the expectable variance in cohort studies, making it easier to estimate the ideal group size in future clinical studies. Furthermore, TVB can be used to explore which factors actually cause commonly observed disturbances in the network topology of disorders like dementia or other neurological and psychiatric disorders.

2.2.3.3 Multivariate analysis methods

Multivariate analysis approaches and multimodal integration are the newest way of looking at complex data structure. Project partner Vince Calhoun is one of the driving forces behind developing and applying independent component analysis to extract also hidden information and neuronal network patterns from the data, as already described in section 1.1.2. Joint ICA, in addition, is a way of multimodal integration, like fMRI and structural information, as repeatedly demonstrated by the PI[49-51]. In short, such a joint ICA performs ICA on the different modalities and searches of significant correlations between the individual component weightings. Thereby, significant dependencies between modalities may be discovered that are non-significant when analyzing each modality alone. Within the project, this framework will not only be applied but also extend, together with project partner Vince Calhoun, by including deep-learning strategies.

2.2.3.4 Reliability measurements

The PI of this project was among the first researchers who introduced the intra-class correlation coefficient (ICC) in neuroimaging more than a decade ago[6,52]. The proposed project will continue on that path and extend the previous work by developing new and innovative methods of reliability measures. Since its first application, the ICC has become widely accepted as an indicator of reliability in the field of neuroimaging. Using ICCs [6,53] brings the advantage of being able to differentiate between sources of variance, and hence explore both within-subject, between-subject, and inter-rater variations. One task of the present project is to develop a method that allows controlling the "rater" dependent variability more efficiently, which in case of neuroimaging means improving the measurements conducted with different MR scanners. This will provide an estimate of scanner dependent sources of variations, which then will be fed into the different model estimations and simulations. This will be supplemented by a regular quality assessment of the used MR equipment, using phantom measures and standardized protocols. These measures will serve as additional, underlying sources of variability in the analysis pipelines. Further, a revised formulation of the ICC will be developed that will allow inferences on intra-individual reliability measures within the group of participants from the longitudinal study. The aim of this is to infer whether the inclusion of certain parameter will increase the reliability. This requires a further split of the variance components.

2.2.4 Next-generation data analyses

As outlined above, current analysis strategies mainly rest on the examination of the BOLD signal alone and might be easily affected by other parameters that are not directly related to the neuronal activity. Hence, new analysis strategies have to be developed, which is one of the main tasks of this project, in particular of SP3.

2.2.4.1 Bayesian statistics

In recent years, Bayesian statistics have become an important method within the field of neuroimaging. By using Bayesian statistics one is able to estimate the probability of certain outcomes rather than describing deviations derived from a null hypothesis. In general, Bayesian statistics rely on the implementation of realistic priors that can be used to make predictions of possible outcomes, hence to minimize prediction errors and to increase "Bayesian" believes. As mentioned above, the DCM method already rests on the Bayesian framework, and there are tools available that use Bayesian inferences also in neuroimaging statistic[54]. However, they are rarely used and not suitable for rs-fMRI. Thus, the present project will go beyond the current state-of-the-art by developing a rs-fMRI analysis tool that operates with realistic priors for network identification that stem from real measurements, topological measures (see below), as well as TVB simulations. The development of realistic and robust spatial and temporal/spectral priors is the foremost aim of this project (mainly SP3). Such a new analysis strategy and software tool will be applicable not only for rs-fMRI but also for "classical" task-related fMRI, and, as an ultimate goal, in a clinical context on an individual subject basis without the need of group studies, which is mandatory with current tools.

2.2.4.2 Deep learning

Deep learning is one of the new "big data" analysis procedures. The main characteristic that differentiates deep learning from other classifier approaches, e.g. for identifying sub-populations in a multimodal data space, is that features are learned automatically and do need a feature selection as a preceding step, which

removes subjectivity and substantially improves accuracy[55]. Deep learning has shown superior performance in detecting cross-modality relations and has attracted a substantial amount of attention among researchers from various fields. Further, it has been nominated as one of the "10 breakthrough technologies" by MIT Technology Reviews (https://tinyurl.com/zx82sg5). In the present project, deep-learning strategies will be used to address the question whether intra-individual data will be classified in the same way across time points, i.e. does a deep learning discover the (presumably) constant neuronal feature and disregard the varying (vascular) feature from the BOLD signal. Further, does deep learning reliably identify individuals in the multidimensional data space consisting of the longitudinal and cohort study? And, finally, does it differentiate between patients and healthy control subjects (SP5)?

2.2.4.3 Topological data analysis (TDA)

The purpose of topological data analysis is to simplify high-dimensional and complex data in a way that preserves only the essential information[56]. This project aims at applying topological data analysis on brain imaging data as a tool for classification in the sense of machine learning.

Similar to deep learning, and in contrast to DCM and TVB, this approach is purely data driven with no prior assumptions or underlying model of the brain. More precisely, divisive covers[57] will be used as classifiers on patients level. The purpose is to integrate the analysis of experimental and clinical data by using the clinical data as labels. A first attempt at applying divisive covers directly for classification on standard machine learning data sets has shown promising results.

Presently, topological algorithms are mainly used for unsupervised machine learning[58]. We will further develop the use of these methods for exploration and visualization of brain imaging data. The underlying idea is that data has a shape, and that this shape can be visualized. For classification, persistent homology is used as a preprocessing step before using techniques from supervised machine learning[59].

Our approach is self-contained in the sense that it does not rely on third-party classification tools, and it avoids the resource intensive computation of persistent homology.

2.3 Research objectives, hypotheses, and questions

In the following, specific hypotheses (H) and tasks (T) are defined for the different subprojects

2.3.1 Subproject 1 (SP1)

H1.1: High inter-individual variability in the open-access databases

T1.1: Analyse inter-individual variability for participants in the age range 20-30 and estimate sample size and effect size for SP2 and SP4

H1.2: Inter-individual variability of the BOLD signal is higher than the variability in network dynamics

T1.2: Analyse BOLD signal variability; specify DCM models; analyse variability of DCM parameter estimates; analyse brain network dynamics (e.g. DMN vs. EMN); create first TVB models

2.3.2 Subproject 2 (SP2): Reliability of longitudinal and cohort multi-scanner rs-fMRI

H2.1: There will be high reliability on the group-level but a low to medium reliability of rs-fMRI networks on the inter- and intra-individual level, with substantial time and scanner factor.

T2.1: Conducting a longitudinal rs-fMRI study; analysing BOLD signal variability, analysing inter- and intra-individual variability in brain network dynamics (e.g. DMN vs. EMN); analysing reliability of DCM parameter estimates; continue with TVB models; apply ICA and deep learning

H2.2: Comparable inter-subject variability at T1, within the cohort rs-fMRI study and SP1

T2.2: Compare inter-subject variability across studies (>1000 participants) and extract systematic patterns of variability and stability (DCM parameter, network dynamics, ICA, TDA, etc.)

Table 1	T0	T1 (Winter, morning)	T2 (Summer, daytime)	T3 (Winter, daytime)	T4 (Summer, evening)	T5 (Winter, evening)	T6 (Summer, daytime)	T7 (Winter, daytime)	T8 (Summer, morning)
Neuropsychology	X			X			X		
BMI, tea, coffee, HRV, etc.	X	X	X	X	X	X	X	X	X
Rs-fMRI & EEG		GE	Siemens	Siemens	GE	GE	Siemens	GE	Siemens
MRS		X	X	X	X	X	X	X	X
Structure & DTI		X	X	X	X	X	X	X	X
ASL		X	X	X	X	X	X	X	X
Hormones	X	X	X	X	X	X	X	X	X
MEG(if available)		X	X	X	X	X	X	X	X
Predictive Neurocomputation			x	x	x	x	x	X	X

The table outlines the study protocol that will be followed by each participant of the longitudinal/cohort study. The order of the different measures will be individually randomised. "Winter" refers to dark periods and "Summer" to light periods of the year in Norway/Bergen, which will not be strictly identical with the metrological definitions. The time T0 is defined as the study inclusion point, where the neuropsychological testing and the first assessment of hormones will be made. The latter one will be used as an initial baseline to verify that the scanning situation does not change the level of hormones (e.g. cortisol). At each time point (T0-T8), the participants will deliver two saliva samples, one at the beginning and one at the end to reduce measurement errors. Saliva samples will be analysed in the bio-lab at the Department of the PI. Further, BMI, HRV, blood pressure, consumption of nicotine, alcohol, caffeine, and tea will be recorded (T0-T8).

2.3.3 Subproject 3 (SP3): Neuroinformatics: New analysis strategies for analysing rs-fMRI

H3.1: Rs-fMRI analysis can be improved through Bayesian-based statistics that use priors that are independent of the BOLD signal, like spectral characteristics or spatial patterns

T3.1: Get data from SP1 and extract spatial patterns and spectral characteristics; estimate variability and create priors from them. Apply new algorithm to SP2 and SP4

H3.2: Topological data analysis is a new, more efficient and objective measure of rs-fMRI activity

T3.2: Develop/improve algorithm for topological data analysis; apply algorithm to data from SP1, SP2, SP4; compare results with DCM, ICA, and Bayesian approach; test algorithm on TVB models for detecting simulated features.

2.3.4 Subproject 4 (SP4): Endogenous and exogenous influences on rs-fMRI reliability

H4.1: Endogenous and exogenous parameter will influence BOLD signal and neuronal activity (Fig.1)

T2.2: Analyse influence of endogenous and exogenous parameter on all measures of inter- and intra-individual variability, extracted by SP2 from the longitudinal and cohort study; identify those that influence BOLD signal and neuronal activity and quantify their influence; identify those that may affect reliability measures; adapt intra-class-correlation (ICC) reliability measures

H4.2: The Balloon model can be improved by including these endogenous and exogenous parameters

T4.2: Revise Balloon model and test improved DCM models

2.3.5 Subproject 5 (SP5): Brain network dynamics in patients suffering from Parkinson's Disease:

H5.1: Patients with PD show altered network dynamics and connectivity patterns, identifiable on the single case level, using the improved and new developed rs-fMRI analysis strategies

T5.1: As proof of concept, extract data from an ongoing examination of PD patients and apply the developed methods and outcomes to this data set on a group and single subject level; deep-learning classifications, TDA, DCM and ICA models will identify PD

2.4 Project plan

As outlined in details in the previous section2.3, in Figure 2, and the milestones in the electronic grant application form, the project consists of four dependent and interacting projects (SP1-4), and one proof of concept subproject (SP5)(see also section 2.8 for risk evaluation). The projects will start in a hierarchical order, with SP1 being first since it has to deliver sample size estimations for SP2. SP2 and SP4 will run in parallel, while SP3 is independent of SP2 and SP4 in the initial phase and may start in between SP1 and SP2/4 since SP2/4 are dependent on the method developments in their later phase.

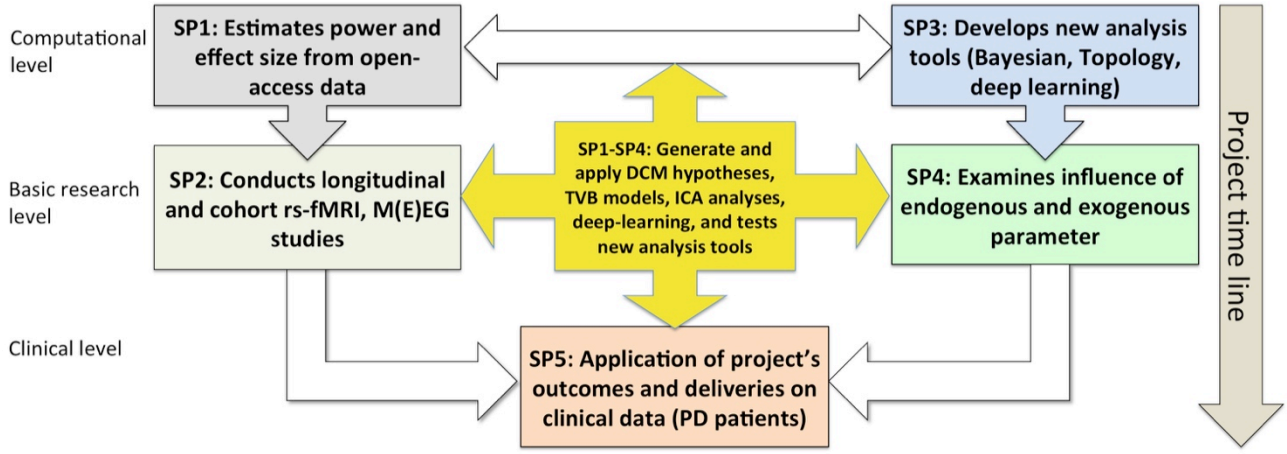

Figure 2: Project and subproject (SP) organisation and interactions: Each SP has its own core aspect, and SP1-SP4 work jointly together on aspects like data modelling and simulation, deep-learning procedures, or improvements of software and theoretical

models. This joint activity (yellow box) is the core of the project and anchor point for the external project partners. The ultimate test of the outcomes of this project will be the application to clinical data (SP5) towards the end of the project.

The PI will ensure that results, resources, and information will flow between projects. This means, the evaluation of the first-wave examination of participants (SP2) will inform the analyses of the neuroimaging data and will then be channelled into the neurocomputational models (SP3). In return, results of SP3 will be made available to SP1, SP2 & SP4 to help identify possible sources of variability.

2.5 Project management

This is an interdisciplinary project that will only be successful through intensive communication and regular exchange. Therefore, project members will meet regularly, once per year as meetings of the entire research group, including the international partner, and in between through small group meetings, lab visits, and telephone/internet meetings. Further, the start phase of the project will be dominated by extended lab training in the respective labs of the international project partner, with follow-up meetings during the entire project period. The local and national partner will meet more often; on average once or twice per month.

2.5.1 Principle Investigator

Prof. Karsten Specht is employed as professor at the Department of Biological and Medical Psychology. He is co-head of the Bergen fMRI group and head of the "Bergen Research group on auditory perception" (BeRG-AP) that has a focus on auditory processing, speech comprehension, developmental speech and language disorders, rehabilitation from aphasia, as well as music processing and music therapy in neurological disorders. Further, he is director of the International Graduate School in Integrated Neuroscience (IGSIN) and deputy head of the Department of Biological and Medical Psychology. He has a background in physics and cognitive neuroscience, and a more than 20 years long experience in the field of neuroimaging. He has an international network of collaborators, and he was among the few researchers who used functional magnetic resonance imaging already in mid-1990th, and he was the first who introduced the intra-class correlation coefficient as a measure of reproducibility in the field of neuroimaging. Over the years, he has worked not only on the application of functional imaging in clinical and basic research studies but also on the multimodal integration of data sources, such as combining fMRI with EEG or MRS. His broad spectrum of interest and competence is also reflected in the number of finished bachelor (n=9), master (n=22) and PhD (n=11) thesis of students coming from the field of psychology, neuroscience, or logopedics, where he acted as main- or co-supervisor.

2.5.2 PhD candidates

The three PhD candidates (2 NFR/1 UiB) will have a background in psychology, (cognitive) neuroscience, or (neuro-) informatics or related fields from natural sciences. It will be mandatory for the candidates that they have or learn basic programming skills in order to conduct the complex analysis pipelines, required by this project. One PhD candidate will be assigned to project SP3 (neuroinformatics) and requires additional skills in mathematics and/or topology, the other PhD candidates will be assigned to project SP2&4, respectively.

2.5.3 Postdoctoral fellow

The two postdoctoral fellows will act as co-supervisor for two of the PhD candidates. The candidates for these positions will have a background in cognitive neuroscience and neuroinformatics, respectively. The postdoctoral fellow will be mainly associated to the subprojects SP1, SP2, and SP5, and through co-supervision of the respective PhD candidate to subproject SP3 & SP4.

2.5.4 Project manger/research assistant

A research assistant will act as project manger, coordinating the different project with respect to scanner time (SP2/4), organising research subjects, keeping contact with participants (SP2), and keeping contact with the international project partner, in particular with their respective project manager.

2.5.5 Professor II

A professor II position will be associated with this project with teaching obligations at IGSIN. Preferably, one of the project partners will fill this position, or, alternatively, an expert in clinical fMRI.

2.5.6 Project partners

The research group will be an interdisciplinary team that comprises besides the local group members also national and international project partner. These project partners are highly respected researchers and they are the leading experts in the field of neuroscience and neuroimaging. These experts, together with their research groups, will substantially support this interdisciplinary project through extensive lab visits, training courses, and continuous exchange of data, models, algorithms, and software updates. Regular meetings of project partners will take place once a year, with interspersed Skype-meetings on demand, as well as at conferences.

2.5.6.1 National partner

Assoc. Prof. Morten Brun, Dept. of Mathematics, University of Bergen. Brun is expert in topological data analysis, and part of the topology group at the Dept. of Mathematics. Brun will be responsible for the subproject SP3 and he will act as co-supervisor for the respective PhD candidate.

Gaute Einevoll, Faculty of Science and Technology, Norwegian University of Life Sciences, is professor in physics and expert in computational neuroscience. He is co-founder of the Norwegian node of the International Neuroinformatics Coordinating Facility (INCF). He will mostly contribute to SP3 and to all aspects related to neuroinformatics and computational neuroscience, which goes through all projects.

2.5.6.2 International partner

Prof. Karl Friston, Wellcome Trust Centre for Neuroimaging, University College London, is the inventor and core developer of SPM and DCM and an expert on Bayesian statistics. He has worked in the field of neuroscience since the early 1990th and recently received the glass-brain award for his live achievements in the field of cognitive neuroscience and neuroimaging. Prof. Friston will act as advisor for the development of DCM models (SP2, SP4) and new Bayesian tools (SP3). Through lab visits and presentations, he and his research group will be important supporters and advisors to this project throughout the entire project period. This collaboration will be a continuation of a several weeks lasting lab-visit in 2016 as part of the sabbatical of the PI, and a joint publication is in preparation.

Prof. Viktor Jirsa, Director of Research at the Centre National de la Recherche Scientifique (CNRS) in France and Director of the Institut de Neurosciences des Systèmes (UMR1106 Inserm) at Aix-Marseille University, is co-developer of the neuroinformatics platform The Virtual Brain (www.thevirtualbrain.org). Prof. Jirsa will be the main contact person with respect to the implementation and development of TVB models for this project. Prof. Jirsa and the TVB core team will be responsible for the training of project members in the implementation of TVB and developing of TVB models. Support will be given through extended lab visits in Marseille and participation in dedicated meetings and training seminars of the neuroinformatics and TVB community. Prof. Jirsa will be mostly involved in SP1, 2 & 4.

Prof. Vince Calhoun, The Mind Research Network and the University of New Mexico, is the core developer of software tools that rest on independent component analysis (ICA). In the present project, Prof. Calhoun will be supporter for the subproject on multivariate and multimodal integration, using methods such as joint ICA, as well as its implementation and combination with deep learning strategies. Prof. Calhoun and the PI have also previously worked on related fields, as indicated by shared publications [36,60]. Prof. Calhoun will be mostly involved in SP2 & 3, in particular in relation to deep-learning and multivariate strategies.

2.6 Research and teaching environment

The Department of Biological and Medical Psychology, University of Bergen, is an international and multidisciplinary research environment. The department has a biochemical lab, four electrophysiology labs with shielded chambers equipped with EEG systems with up to 96 channels, one audiolab, and one TMS lab. Further, an application has been sent for a MEG system but a final decision is pending at the time of writing this proposal. In addition, the research group of the PI has access to two 3T MR systems (GE 750 and Siemens PRISMA) at the Haukeland University hospital, which can be used at the expense of 4000 NOK that cover the costs for the use of the scanner, the two radiographers, and the evaluation of the images by a radiologist (see budget). The department has its own biochemical lab, where the saliva samples will be analysed to extract concentrations of cortisol, estradiol, progesterone and testosterone.

The PhD students will be enrolled at the International Gradual School in Integrated Neuroscience (IGSIN), which is headed by the PI of this project. IGSIN provides courses in neuroscience, fMRI methods, statistics, and administers other PhD courses. IGSIN is also collaborating with the National Research School in Neuroscience (NRSN), and PhD candidates will be registered here, as well, in order to receiving additional support and funding for course attendance outside of Bergen.

2.7 Dissemination plan

Please see the information in the electronic grant application form.

2.8 Risk evaluation

The project can be categorised as a high-risk high-gain project. A success of this project will have a significant influence on the entire field, not only due to the collaboration with the leading persons of the field, but also due to the substantial increase of knowledge on the effect of certain parameter on the reliability and variability of fMRI studies. By know, there is no study that has studied this in such a systematic way as the present project. However, the major risk of this project is in failing in identifying

sources of variability. On the other hand, this would be an advance in our knowledge, as well, since the most likely sources will be examined.

Due to regular meetings of the project members (see section 2.5), risk evaluation will be constantly performed, followed by timely adjustments. Adjustments may contain increase of sample sizes, collecting additional or other parameter (hormones, etc.), inclusion of other open-access databases, or adjustments in the analysis pipeline, setup of models, or algorithms.

3 Budget

Please see the information in the electronic grant application form.

4 General considerations

4.1 Environmental impact

Not applicable

4.2 Ethical perspectives

The project plan, the informed consent and all invitational letters will be sent to the regional ethic committee (REK) for approval. All sensitive data will be stored and processed in stationary, locked network systems, not accessible from the outside. Only anonymised data will be processed. Before data are shared with collaborators, clearance from REK and the national service for data security (NSD) will be obtained. The data from the neurocomputing subproject are completely anonymous.

The selected neuroimaging methods are without any health risk. However, it has to be clarified whether a participant can enter the MR scanner, and clear exclusion criteria are defined. The Bergen fMRI group has more than 20 years of experience without any incidents. All MR scans will be evaluated by a radiologist, and participants will be informed and called back for further examinations in case of suspicious findings. This is part of the standard routines of the Bergen fMRI group. The study protocol, informed consent, and study outline will be sent to the regional ethic committee for approval. The protocol will define clear exclusion criteria and will state clearly that any participant is allowed to leave the project at any time without any consequences.

4.3 Gender issues (Recruitment of women, gender balance and gender perspectives in research)

Gender issues apply for both, the project staff as well as the examined participants. With respect to the project staff, it is a clear aim that both genders are equally represented among the project members, which is also in accordance with the agenda of the University of Bergen. Due to its interdisciplinary and international character this project will provide a profound basis for a successful career in research, especially in the light of a skewed gender distribution in higher positions, with substantially more men than women.

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