Analysis plan for the project "Assessing the relationship between sub-clinical anxiety and resting-state fMRI"

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1 Collaborators

This project is being conducted in collaboration with the following individuals:

- Dr Olivia Harrison, University of Otago (Primary Supervisor)
- Associate Professor Bruce Russell, University of Otago (Co-supervisor)
- Professor Paul Glue, University of Otago (Co-supervisor)
- Professor Kyle Pattinson, University of Oxford (Collaborator)

2 Foreword

This document contains an analysis plan for the project entitled "Assessing the relationship between sub-clinical anxiety and resting-state fMRI". The project concerns the analysis of resting-state functional magnetic resonance imaging and measures of trait anxiety via State-Trait Anxiety Inventory (STAI) scores in healthy volunteers collected by the Breathe Oxford Team at the University of Oxford [1].

The project contains data of forty healthy, right-handed volunteers (20 males, 20 females; mean age ± SD , 25.7 ± 7.3 years, Inter-Quartile Range (IQR) 5.5). For these participants their State-Trait Anxiety Scores (Trait component only) (STAI-T scores) (mean 30.2 ± 6.3, IQR 9.5) and Anxiety Severity Index (ASI) scores (mean 14.8 ± 7.0, IQR 8.0) were collected and are of primary interest in the present study. The wider data for this study included questionnaire data using the Center for Epidemiological Studies Depression Scale scores (CES-D scores), State-Trait Anxiety Scores (State component) (STAI-S scores), results from a breathing-related conditioning task, as well as the associated behavioural, physiological, and structural and functional MRI data, measured on a 7 Tesla (7T) scanner by Breathe Oxford. The original study also included a hypercapnic reactivity test and minimal exercise test.

The purpose of this analysis plan is to provide a brief background for the proposed work, formulate the research question(s), explicitly describe planned and potential post-hoc analyses, and give the rationale for each analysis step. All code created over the course of the project will be version-controlled and documented on the internal GitHub page (https://github.com/IMAGEotago) of the IMAGEotago research group.

3 Introduction

Anxiety is a psychological and physiological state characterised by cognitive, physiological, and behavioural components. It is described as the apprehensive anticipation of future danger or misfortune and is accompanied by a feeling of worry, distress, and/or somatic symptoms of tension [2]. Although anxiety is a natural adaptive reaction, when it is greater than what would be expected for a given situation, it becomes pathological and can cause stress and interfere with the ability to cope successively with life challenges [3, 4]. Anxiety disorders, classified by the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V), differ from healthy fear or anxiety by being excessive or persistent, and are among the most common psychiatry disorders, with a lifetime prevalence of 24.9% in New Zealand [5].

Whilst anxiety is primarily diagnosed by behavioural properties, neuroimaging can help us to understand the underpinnings of these behaviours. In particular, resting-state functional magnetic resonance imaging (fMRI) measures spontaneous low-frequency fluctuations (<0.1 Hz) in the blood oxygen level-dependent (BOLD) signal, and can allow us to investigate the functional architecture of the brain [6]. First described by Biswal et al in 1995, resting-state fMRI is an imaging technique that is playing an increasing role in characterising normal and abnormal functional brain connectivity in a variety of clinical conditions [7, 8]. Spatially distinct brain regions with correlated activity, forming organised networks known as resting-state networks, can be detected by using the relative temporal changes in the BOLD signal [7].

It has been suggested that anxiety disorders arise out of potential abnormalities in cortical-subcortical interactions, resulting in an inappropriate expression of the fear response seen in anxiety [3]. Previous studies of anxiety disorders have concentrated on the amygdalo-cortical circuitry; yet evidence suggests that the amygdala, anterior and posterior cingulate cortex, medial prefrontal cortex, insula and hippocampus all play a role in anxiety disorders [9]. There is also limited literature on the association between resting state brain functional connectivity, as studied using resting state fMRI, and reported anxiety levels in the sub-clinical population [10]. Individuals with high trait anxiety in these sub-clinical populations are at an increased risk to develop both depression and anxiety disorders [10].

Since resting-state fMRI is acquired in the absence of a stimulus or task, the observed brain activity is not altered by specific tasks as in task-based fMRI [7]. This ability to map brain function by observing brain signals during rest is useful, particularly in the sub-clinical population. The lack of task means that resting-state fMRI can be used more easily within a clinical environment to assess the neural circuitry of patients as it does not require stimuli to be presented to the individual nor does it require them to respond to the stimuli [8]. This is especially useful with certain populations who may have difficulty with the task instructions or performing the task [7].

A better understanding of the neural circuitry involved in anxiety in both healthy and clinical populations will provide a basis for understanding how the nervous system regulates this emotional process and the neural pathways involved. It will also provide important clues for beginning to understand the basics of individual differences in anxiety that exist in both the normal and abnormal range [11]. It also may aid in developing and determining appropriate treatment strategies that target

abnormal function in anxiety disorders [10].

4 Research questions

- ***Experimental Question 1:*** *Are there differences in amygdala functional connectivity across levels of anxiety and/or anxiety sensitivity within a healthy population?*

  Hypothesis: There will be altered functional connectivity between the amygala and other regions of the brain for different levels of anxiety, with different connectivity patterns on each side of the brain [12–15]. There may also be less distinct connectivity patterns of the amygdala sub-regions with increasing levels of anxiety [16].

- ***Experimental Question 2:*** *Are there differences in resting state networks across levels of anxiety and/or anxiety sensitivity within a healthy population?*

  Hypothesis: There will decreased functional connectivity of the default mode network, which has been suggested to be involved in episodic memory and self-projection [10, 17, 18].

5 Exploratory research questions

In this analysis we will also use the opportunity to investigate the following exploratory questions:

- ***Exploratory Experimental Question 1:*** *Are there differences in functional connectivity of additional targeted brain regions across levels of anxiety and/or anxiety sensitivity within a healthy population?*

  We will conduct a set of exploratory analyses to assess the functional connectivity of the following brain regions across levels of anxiety and/or anxiety sensitivity within a healthy population: frontal medial cortex, insula cortex, hippocampus, parahippocampus, anterior cingulate gyrus, posterior cingulate gyrus.

- ***Exploratory Experimental Question 2:*** *Are there differences in resting state networks identified in addition to the default mode network across levels of anxiety and/or anxiety sensitivity within a healthy population?*

  We will conduct a set of exploratory analyses to assess the functional connectivity of the resting state networks identified by PROFUMO in addition to the default mode network across levels of anxiety and/or anxiety sensitivity within a healthy population.

6 Dataset

The data is of forty volunteers (20 males, 20 females; mean age $\pm$SD, $26\pm$ 7 years), recruited from the general Oxford community via study advertisements. Participants were healthy controls with no history of smoking or any respiratory disease.

Participants were excluded following an inability to comply with experimental protocol, or a contraindication to magnetic resonance imaging at 7T. The participants were evenly split into two groups, those who regularly participated in endurance sport and 20 age- and sex-matched (± 2 years) sedentary participants. Imaging was performed using a 7T Siemens Magnetom scanner with 70 mT/m gradient strength and a 32 channel Rx, single channel birdcage Tx head coil (Nova Medical). A T2*-weighted, gradient echo EPI was used for functional scanning. The field of view (FOV) covered the whole brain and comprised 63 slices (sequence parameters: TE, 24 ms; TR, 3 s; flip angle, 90°; voxel size, 2 x 2 x 2 mm; field of view, 220 mm; GRAPPA factor, 3; echo spacing, 0.57 ms; slice acquisition order, descending; orientation, axial tilted), with 190 vol (scan duration, 9 min 30 s) for the resting-state acquisition (eyes open). A T1-weighted structural scan (MPRAGE, sequence parameters: TE, 2.96 ms; TR, 2200 ms; flip angle, 7 °; voxel size, 0.7 x 0.7 x 0.7 mm; field of view, 224 mm; inversion time, 1050 ms; bandwidth, 240 Hz/Px) was acquired and used for registration of functional images. Fieldmap scans (sequence parameters: TE1, 4.08 ms; TE2, 5.1 ms; TR, 620 ms; flip angle, 90°; voxel size, 2 x 2 x 2 mm) of the B_0 field were also acquired to assist distortion correction.

Image processing was performed for each image using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library (FMRIB, Oxford, UK; FSL version 5.0.8; http://fmrib.ox.ac.uk/fsl/). The following preprocessing methods were used: motion correction and motion parameter recording (MCFLIRT), spatial smoothing using a full-width half-maximum Gaussian kernel of 2 mm (to maintain the resolution afforded by 7 T scanning, and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting; 120 s). B_0 field wrapping was conducted using a combination of FUGUE (FMRIB's Utility for Geometrically Unwarping EPIs) and BBR (Boundary-Based-Registration; part of FEAT: FMRI Expert Analysis Tool, version 6.0). Data denoising was conducted using a combination of independent components analysis (ICA) and retrospective image correction (RETRO-ICOR) using the externally recorded phsyiological measures, and this also included simultaneous regression of motion parameters. Noise components from the ICA decomposition were identified via established noise properties pertaining to spatial location, frequency and timeseries patterns.

For more details on study procedures unrelated to the resting-state functional imaging, please see the paper [1].

7 Exclusion of datasets

No participants or data was excluded from the analysis.

8 Analysis procedures

8.1 Preprocessing and Registration

Image processing will be performed for each image using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library (FMRIB, Oxford, UK; FSL version 5.0.8; http://fmrib.ox.ac.uk/fsl/). Non-brain structures (skull and surrounding tissue) will be removed from the T1-weighted structural

scans using BET. Following this preprocessing step, the scans will be registered to the MNI152 (2 x 2 x 2 mm) standard space (average T1 brain image constructed from 152 normal subjects at the Montreal Neurological Institute (MNI), Montreal, QC, Canada) using a two-step process: 1. Registration of subjects' whole-brain EPI to T1 structural image will be conducted using BBR (6 DOF), and 2. Registration of the subjects' T1 structural scan to 2 mm standard space will be performed using an affine transformation followed by nonlinear registration (FNIRT).

8.2 Functional Connectivity of the Amygdala: Experimental Question 1

This analysis will be conducted using the seed-based correlation analysis (SCA) method. We will firstly spatially define the left and right basolateral and centromedial amygdala nuclei seed ROI using uniformity test map created by the automated Neurosynth meta-analysis of the term 'anxiety' and masking it with the Juelich Histological Atlas in FSLeyes, before creating a binary mask and transforming it into functional space. We will then extract the mean timeseries from the ROIs in each subject and create a seed-based connectivity map for each subject by running a first-level FEAT analysis. These t-statistic subject-wise correlation maps will be transformed to Z-scores before conducting group-level analysis of the voxel-based maps using FEAT. At the group level, one general linear model (GLM) will include STAI scores, plus a gender regressor, as well as the interaction between gender/STAI. The contrasts of interest for this experimental question are positive and negative contrasts on the STAI score regressor, the gender regressor, and the interaction. A seperate GLM at the group level will include ASI scores, plus a gender regressor, as well as the interaction between gender/ASI. The contrasts of interest for this experimental question are positive and negative contrasts on the ASI score regressor, the gender regressor, and the interaction. We will use non-parametric permutation testing via the randomise tool in FSL, combined with threshold-free cluster enhancement. We will use a significance threshold of $p < 0.05$, and constrain inference and family-wise error correction for multiple comparisons to a grey matter mask within the brain.

8.3 Default Mode Networks: Experimental Question 2

The default mode network will be identified from the Probabilistic Functional Modes (PFMs) inferred using PROFUMO (version 0.11.3; https://git.fmrib.ox.ac.uk/samh/profumo). We will constrain the analysis to within a liberal whole-head mask. We will use the same thresholding and inference parameters as those listed above in Experimental Question 1.

8.4 Functional Connectivity of Other Regions: Exploratory Experimental Question 1

The seed ROIs will be selected from the brain regions displayed on the uniformity test map created by an automated Neurosynth meta-analysis of the term 'anxiety'. We will spatially define each seed ROI using the Neurosynth results masked by the

Harvard-Oxford Subcortical or Cortical Structural Alases in FSLeyes, before creating a binary mask and transforming it into functional space. For each ROI, we will then extract the mean timeseries from ROI in each subject and create a seed-based connectivity map for each subject by running a first-level FEAT analysis. These t-statistic subject-wise correlation maps will be transformed to Z-scores before conducting group-level analysis of the voxel-based maps using FEAT. One GLM will include STAI scores, plus a gender regressor, as well as the interaction between gender/STAI. The contrasts of interest for this experimental question are positive and negative contrasts on the STAI score regressor, the gender regressor, and the interaction. A separate GLM will include ASI scores, plus a gender regressor, as well as the interaction between gender/ASI. The contrasts of interest for this experimental question are positive and negative contrasts on the ASI score regressor, the gender regressor, and the interaction. We will use the same thresholding and inference parameters as those listed above in Experimental Question 1.

8.5 Resting State Networks: Exploratory Experimental Question 2

The Probabilistic Functional Modes (PFMs) will be inferred using PROFUMO and we will constrain the analysis to within a liberal whole-head mask. We will use the same thresholding and inference parameters as those listed above in Experimental Question 1.

9 Revisions

This is version 1 of this analysis plan.

References

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