Analysis plan for the project 'The relationship between interoception of breathing, anxiety and resting-state functional brain connectivity

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1. Collaborators

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

- Doctor Olivia Harrison, University of Otago (Primary Supervisor)
- Associate Professor Bruce Russell, University of Otago (Co-Supervisor)
- Professor Klaas E. Stephan, University of Zurich and ETH Zurich (Collaborator)
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2. Foreword

The following document details the analysis plan of the project entitled 'The relationship between interoception of breathing, anxiety and resting-state functional brain connectivity'. The aim of this project is to ascertain whether/to what extent resting-state functional connectivity (rsFC) in the brain differs as a result of breathing-related interoceptive abilities and/or trait anxiety levels. To explore these relationships, data from 65 healthy volunteers who participated in a breathing perception study at the Translational Neuromodelling Unit (TNU) at the University of Zurich and ETH Zurich will be utilised. Specifically, resting-state brain imaging data, the results of the Generalised Anxiety Disorder Questionnaire (GAD-7) and the output of a breathing-

related perceptual sensitivity and metacognitive task will be central to the described investigation.

The following analysis plan will highlight the rationale for this project, the ways in which the data will be analysed to address a set of pre-determined research questions, as well as how the findings of this research could come to advance our knowledge in this area. Any changes to this analysis plan will be documented in detail on the GITLAB for the IMAGE Otago group.

3. Introduction

Anxiety is both a symptom of common mental health disorders, and a normal response to stressors (Tovote et al., 2015). Although an anxiety response functions to protect us from potential threats, persisting anxiety symptoms can impinge on normal functioning and hinder one's ability to thrive in everyday life (Everly & Lating, 2019).

Interoception is the process through which one perceives their internal bodily states, and some aspects of interoceptive processing have been found to be altered in individuals with higher levels of anxiety (Harrison et al., 2021; Sherrington, 1952). Recently, it was highlighted that individuals with moderate levels of anxiety had poorer perception of an inspiratory resistance and lower confidence in their perceptual decisions relative to those with low levels of anxiety (Harrison et al., 2021). The mechanism by which breathing-related interoceptive abilities and anxiety are related, however, is not yet clear.

Neuroimaging can help us investigate the neural underpinnings of many behavioural outcomes and could be vital in understanding the biological basis of a heightened anxiety response. A resting-state functional magnetic resonance imaging (rs-fMRI) scan, for example, measures the temporal co-activation of brain regions while the recipient is at rest (awake, with their eyes open but not completing a task) (Biswal et al., 1995). The output - known as rsFC - provides information regarding the areas of the brain which are communicating with one another when not engaged in a task (Biswal et al., 1995).

Interestingly, higher levels of anxiety have been linked to changes in the rsFC of the brain, most notably in relation to the amygdala – a brain structure believed to be central in stress and anxiety processes (Baur et al., 2013; Geng et al., 2016). Therefore, the aim of the present project is to investigate the relationship between breathing-related interoceptive abilities, rsFC and anxiety levels in a healthy population. This investigation will help explore potential brain mechanisms through which anxiety and breathing-related

interoceptive processes are related, and could therefore reveal new directions for treating anxiety.

4. Experimental Questions and Hypothesis

- ***Experimental Questions 1-4:*** *How does breathing-related interoceptive sensitivity, decision bias, metacognitive bias and metacognitive performance relate to patterns and intensities of the rsFC of the amygdala?*
  Hypotheses 1-4: Patterns and/or intensities of rsFC will differ as a product of one, or multiple aspects of breathing-related interoception.

- ***Experimental Question 5:*** *How do differences in anxiety scores on the GAD-7 Questionnaire relate to patterns and intensities of rsFC?*
  Hypothesis 5: Patterns and/or intensities of rsFC will differ across levels of anxiety as determined by the GAD-7 Questionnaire.

- ***Experimental Question 6:*** *Presuming there is a relationship between anxiety levels and rsFC patterns and intensities – as will be tested in Question 4 - to what extent is this relationship mediated by the different aspects of breathing-related interoceptive ability?*
  Hypothesis 6: Differences in breathing-related interoceptive ability will partially explain the variance (if any exists) in the rsFC across levels of anxiety. Specifically, we predict any variance in rsFC which occurs across trait anxiety scores will reduce when we control for the different aspects of interoceptive ability.

5. Exploratory Experimental Question

- ***Exploratory Question 1:*** *Does the relationship between rsFC and interoceptive abilities differ as a function of gender?*

- ***Exploratory Question 2:*** *Does the relationship between trait anxiety levels and rsFC differ as a function of gender?*

- ***Exploratory Question 3:*** *Does the relationship between trait anxiety levels, rsFC and interoceptive abilities differ as a function of gender?*

6. Datasets

To address the research questions for this project we will use the data obtained from 60 healthy individuals who took part in the breathing perception study at the TNU in Zurich. These participants formed two groups of 30 completed participants based on their scores on the Spielberger State-Trait Anxiety Inventory (STAI). One group of 30 participants with low levels of anxiety (score = 20-25 on the STAI-T) and the other with moderate levels of anxiety (score =>35). These groups were age- and sex-matched, and all participants were pre-screened for MRI contraindications, smoking status, any current or historical psychological or physiological health conditions and handedness. Additional participants who also have complete data for the resting-state data and behavioural measures will also be included (n=65)

Each of these participants underwent a variety of tests and measures, the datasets which will be of particular interest for this project are the output of a rsFC imaging session, the results of an inspiratory resistance interoceptive task – known as the Filter Detection Task (FDT) – and the individuals' levels of trait anxiety as determined by the Generalised Anxiety Disorder Questionnaire (GAD-7).

Although the GAD-7 Questionnaire was originally constructed to assess the extent of Generalised Anxiety Disorder in a clinical setting (Spitzer et al., 2006), it has also been validated as a measure of trait anxiety amongst the general population (Löwe et al., 2008). Consisting of 7 items, this inventory aims to assess the levels of worry and anxiety experienced by the individual. Each item is to be scored on a Likert Scale (0-3), with higher scores indicating more severe/frequent anxiety symptoms. We have chosen this measure of anxiety as it was not used as a pre-screening tool (unlike the STAI-T), allowing us to consider the whole group of participants across a spectrum of anxiety scores.

The FDT involves participants breathing through a device, where breathing filters can be added or taken away across trials to alter the magnitude of resistance. Each trial consisted of a baseline set of three breaths, followed by either three breaths through an additional set of filters or three breaths against an empty (sham) resistance. After each trial they were asked whether they detected a resistance on their breathing (Yes/No) and how confident they were in that decision (1 being not at all confident and 10 being extremely confident). This task therefore measures multiple aspects of interoceptive ability related to the detection of an inspiratory resistance; the first is a measure of

sensitivity which describes the participant's ability to accurately detect an inspiratory resistance; the second measure determines the participant's decision bias, which is their tendency to report the presence of a resistance; and the remaining two measures represent aspects of metacognition – the first is known as metacognitive bias and depicts the overall level of confidence in which they tend to describe their decisions regarding the presence of a resistance; and the final measure is known as metacognitive performance, which portrays the congruence between the participant's confidence and performance on the task.

7. Exclusion of Datasets

All participants with complete resting state data, FDT data and GAD-7 will be used in this analysis.

8. Analysis Procedures

8.1 GAD-7 Questionnaire Analysis

The GAD-7 will be calculated according to its manual, with the final score reflecting the participant's combined answers across the inventory items. The possible scores range from 0-21, with scores of 0-4 indicating minimal anxiety, 5-9 mild, 10-14 moderate and 15+ severe levels of anxiety. Each participant's score will serve as their measure of trait anxiety for the analysis of the experimental and exploratory questions.

8.2 FDT Analysis

As previously described, the four outcome measures of interoceptive ability are obtained during the FDT. Interoceptive sensitivity is quantified as the number of resistance filters at which performance on the task is maintained at 60-85% accuracy, and metacognitive bias is calculated as the average confidence scores across trials. To calculate metacognitive performance and decision bias a hierarchical Bayesian model (HMeta-d) will be utilised (Fleming, 2017) to estimate a decision bias measure ('c') as well as an 'Mratio', which considers metacognitive performance while controlling for underlying task performance. Given the low number of trials in interoceptive experiments, this hierarchical technique helps to improve the accuracy of the single subject measures of metacognitive performance (Harrison et al., 2020).

8.3 Resting-State fMRI Preprocessing

The resting-state data pre-processing and analysis will be performed using FSL version 6 (the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library, Oxford, UK) (Jenkinson et al., 2012). Standard MRI preprocessing of the raw data will be conducted. This will include steps such as brain extraction using BET to remove non-brain structures from the T1 weighted structural images (Smith, 2002), and motion correction using MCFLIRT (Jenkinson & Smith, 2001). Data de-noising will be conducted using independent component analysis with manual classification of noise components, using automatic estimation for the number of components for each participant (Griffanti et al., 2017). The images from each participant's resting-state scan will be registered to their T1 weighted structural scan using the BBR cost function in FLIRT (6DOF) (Jenkinson et al., 2002). Then all images will be registered to 1mm standard space (MNI152), by using FLIRT and FNIRT to perform affine (12DOF) and non-linear (12+DOF) registration (Andersson et al., 2007).

8.4 Resting-State Analysis

To investigate our experimental questions the resting-state data will be analysed using a seed-based correlation analysis (SCA), with the left and right amygdala serving as the primary seeds of interest. To complete this analysis, we will create probable amygdala masks using a standard atlas – Harvard-Oxford Subcortical Atlas - in FSLeyes (Desikan et al., 2006). We will then threshold and binarise the mask before transforming it from standard to functional space for each participant. From here we will use this mask to extract a timeseries from each subject's amygdalae. This time series data will be used as predictors in a separate GLM for each side using FEAT in order to identify other voxels throughout the participant's brain which show correlated activity with those in the seed ROI. The product of this analysis will be individual seed-based connectivity maps for each participant, which will be converted from their t-statistic form to Z-scores using FEAT. A middle-level fixed effects analysis will be performed to calculate the average and any differences in amygdala connectivity between the two sides of the brain. These individual rsFC maps for each participant will be used in higher-level analyses to answer our experimental and exploratory questions – each group level GLM is described below.

9. Analysis of Experimental Questions

- ***Experimental Questions 1-4:*** *How do the measures of breathing-related interoceptive sensitivity, decision bias, metacognitive bias and metacognitive performance relate to patterns and intensities of the rsFC of the amygdala?*

  Experimental questions 1-4 will be analysed by feeding the rsFC data from the subject-level analysis detailed above, into a higher-level GLM analyses, whereby each interoceptive ability is analysed as a predictor of rsFC across participants. Additional exploratory analyses will be performed where each of the interoceptive measures are regressed against rsFC in separate analyses, due to the significant correlations observed between decision bias and metacognitive bias, as well as metacognitive bias with metacognitive performance (Harrison et al., 2020).

- ***Experimental Question 5:*** *How do differences in anxiety scores on the GAD-7 Questionnaire relate to patterns and intensities of rsFC?*

  Experimental question 5 will be tested using a GLM at the group-level. It will utilise the participant's GAD-7 scores as a predictor of their patterns/intensities of rsFC. Ultimately, this will indicate to what extent trait anxiety levels as determined by the GAD-7 are related to the rsFC of the amygdala seed ROI.

- ***Experimental Question 6:*** *Presuming there is a relationship between anxiety levels and rsFC patterns and intensities – as will be tested in Question 5 - to what extent is this relationship mediated by the different aspects of breathing-related interoceptive ability?*

  This experimental question will be tested using a GLM that includes GAD-7 scores alongside the four interoceptive scores as predictors of the patterns and/or intensities of rsFC. This will give an indication as to what extent any differences in rsFC across levels of anxiety can be explained by differing levels of interoceptive abilities.

10. Analysis of Exploratory Questions:

- *Do any of the relationships detailed in experimental questions 1-5 differ as a product of gender?*

These exploratory questions will be tested using the analyses used for the experimental questions (detailed above), but this time including additional gender and interaction regressors. These analyses will determine how/whether any of the investigated relationships differ as a product of gender.

For all analyses we will conduct non-parametric permutation testing through the randomise tool in FSL. A significance threshold of $p<0.05$ will be utilised, as well as constrain inference and family-wise error correction for multiple comparisons to a grey matter mask within the brain.

11. Revisions

This is version one of this analysis plan.

12. References

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