**ANALYSIS PLAN**

Title: Incorporating uncertainty and valence within models of dynamic interoceptive learning to better understand anxiety

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1. **FOREWORD**

This document contains an analysis plan for the project entitled “Incorporating uncertainty and

valence within models of dynamic interoceptive learning to better understand anxiety”.

The wider project involves analysing the link between anxiety and different measures of respiratory interoception in a pilot study of healthy volunteers at the University of Otago. The project involves data gathered from 20 pilot participants with a range of anxiety levels. Here we will focus on the analysis of a breathing-related learning task and the associated behavioural and physiological data.

The purpose of this analysis plan is to provide a brief background for the project, formulate the research questions, explicitly describe planned and potential post-hoc analyses, and give the rationale for each analysis step. All code created for this project will be version-controlled and documented on GitHub (<https://github.com/IMAGEotago/Katja-BLT-code>).

1. **INTRODUCTION**

Anxiety is a potentially debilitating mental health issue that affects a significant number of the population1. In addition to anxiety disorders being the most common type of mental disorder, anxiety can also manifest as a symptom of many other psychiatric and physiological disorders. It is thought that alterations in interoception – the perception of the internal state of the body – are linked to anxiety2, however the exact relationship and underlying brain mechanisms are still unclear. Perception of breathing is a form of interoception that is fairly easy and safe to manipulate, offering the opportunity to gain unique insights into the respiratory domain of interoceptive processing.

The aim of this study is to investigate the relationship between respiratory interoception and levels of anxiety in a healthy population. Specifically, the study will look at different levels of interoception, including perception, learning, and metacognition, and use state-of-the-art computational models to investigate how these relate to anxiety. We will develop an associative learning model (based on the Rescorla-Wagner model3) to incorporate continuous measures, as well as learning rates that differ under different conditions, for example according to the valence of the breathing stimuli. Previous research has found that learning rates are higher when using negative compared to positive stimuli, and this relationship is related to anxiety4,5.

This project will occur within a wider framework of interoceptive research at the School of Pharmacy at the University of Otago. The results of this study will provide a better insight into the relationship between interoception and anxiety, which may be important for developing novel diagnostic procedures and effective treatment options for symptoms of anxiety.

1. **RESEARCH QUESTIONS AND HYPOTHESES**

The aim of the project is to investigate the following main questions and hypotheses:

**Experimental Question 1:** Can we fit continuous response data in an associative learning model instead of binary response data? How do these models compare in both simulations and empirical data fits?

**Experimental Question 2:** Can we extend our model to incorporate differences in learning between positive and negative stimuli?

**Experimental Question 3:** Are there learning differences between positive and negative stimuli?

*Hypothesis 1:* There will be learning differences between positive and negative stimuli, with the learning rate being higher for negative compared to positive stimuli.

**Experimental Question 4:** How do learning rates with positive and negative stimuli relate to anxiety and subjective interoceptive scores?

*Hypothesis 2:* The learning differences between positive and negative stimuli will be more pronounced in individuals with greater anxiety. Specifically, those with higher anxiety will have higher learning rates for negative compared to positive stimuli.

**Experimental Question 5:** How does the average certainty of participant’s responses relate to anxiety and subjective interoceptive scores?

*Hypothesis 3:* Individuals with higher levels of anxiety will have a lower average certainty in their responses.

1. **DATASET**

In order to address the research questions outlined above, we will collect data from 16 healthy volunteers based in Dunedin to act as the pilot cohort. Individuals will be pre-screened for age, level of physical activity, smoking status, colour-blindness, and any history of major physiological or psychological conditions (a full list of exclusion criteria can be found on the [participant information sheet](https://www.otago.ac.nz/pharmacy/research/otago824330.pdf)) before being recruited to participate in the study.

Data will be collected from each participant over the course of a single session. During the session, participants will complete a number of tasks, including filling out a set of questionnaires to assess levels of anxiety (measured using the Spielberger Trait Anxiety Inventory or STAI)6 and other affective dimensions. Participants will also complete a number of tasks to measure interoceptive and exteroceptive perceptual thresholds as well as emotional bias. The task that forms the main focus of this analysis plan is the Breathing Learning Task (BLT). During this task, participants breathe through a facemask that allows an inspiratory resistance to be applied at set intervals. Two visual cues will be paired with a probabilistic outcome of either 80% or 20% chance of experiencing inspiratory resistance. At certain pre-established time points during the experiment, the pairings will be switched so that the cue predicting 80% chance of inspiratory resistance will now indicate 20% chance and vice versa. Participants will be asked to predict the likelihood of an upcoming resistance on a scale of 0-100 based on the cue presented (this provides continuous response data as opposed to a yes/no prediction which provides binary response data).

Participants will complete a total of 80 trials, in addition to 6 practice trials at the start of the experiment. During this task, physiological recordings of inspiratory pressure, breathing rate, breathing volume and heart rate will be taken using a spirometer and pulse monitor.

1. **EXCLUSION OF DATA SETS**

Every data set collected will be carefully checked to ensure its quality. Data sets will be excluded from further analysis for the following reasons:

* The task is not completed
* The task was misunderstood
* More than 10 missed trials
* If binary model fit for the data set is not above chance (as determined by the likelihood ratio test; see section 6.2 below)

1. **BREATHING LEARNING TASK ANALYSIS**

This task includes a combination of physiological and behavioral data which are recorded simultaneously. Each type of data will first undergo separate preprocessing before being analysed in combination.

## Physiological data preprocessing and analysis

### Data preprocessing in LabChart (v8.1.17)

Physiological data will be recorded and preprocessed using dedicated physiological recording software (LabChart 8; ADInstruments Ltd, Oxford, United Kingdom). The following steps will be carried out for preprocessing:

* Calculation of breathing rate and depth from spirometry data
* Calculation of heart rate in beats per minute from the pulse data

### Preparation of physiological data for combined analysis

In addition to recording physiological measure, LabChart will automatically record the start and end of any inspiratory resistance applied, as well as recording the start of the non-resistance trials. These recordings will be used to determine the timings of the resistance and non-resistance trials, as well as the immediately preceding cue periods.

### Physiological summary measures

Maximum and average values will be calculated for each of the following physiological measures for each resistance trial, each non-resistance trial, each cue period and inter-trial-interval:

* Inspiratory pressure
* Breathing rate
* Breathing depth
* Heart rate

## Analysis of Experimental Question 1

In order to fit and analyse the continuous response data, we will first construct an appropriate learning model. As the basis for our perceptual model we will use the Rescorla-Wagner framework; an associative learning model where learning is driven by prediction errors – the difference between predictions and the actual outcomes. This model has previously been successfully used for this type of task using binary response data, paired with a softmax or sigmoid response model to map binary predictions to the perceptual model7. Here, we will firstly adapt this response model to use continuous prediction data. This will thus provide two models to test against each other – one using binary response data (RW-B) and the other using continuous response data (RW-C).

To evaluate the performance of the two models, data from 500 synthetic subjects will be simulated and then assessed for parameter recovery for both models. The synthetic data will be generated using a range of values for (the free parameter in both of the models, representing the learning rate) that are randomly sampled from a uniform distribution with a minimum value of 0.0 and a maximum value of 1.0 (the range of possible values for ), as well as a constant level of noise. Both of the models will then be fit to the synthetic data, and the recovered parameters will be compared with the simulated parameters using linear regression. Parameter recovery will be quantified using Pearson’s correlation coefficient, as well as visual inspection of the simulated and recovered parameter values.

Assuming that both models allow parameter recovery to an acceptable level (simulated and recovered parameters show a significant correlation at *p* < 0.05), the next step will be to fit the data gathered from the 20 participants to both models (continuous responses will be binarized to create the dataset for the RW-B). Learning rates fitted by the models will be compared using a two-tailed t-test with a significance level of *p* < 0.05. For the binary model, we will compare the resultant model fit with chance performance using the likelihood ratio test8. Both models will be taken forward for further investigations.

## Analysis of Experimental Question 2

The next step to developing the chosen model will be to add a parameter for positive and negative stimuli. As was done for the initial models, the updated model will first be run with simulated data in order to check for adequate parameter recovery (following the same procedures as outlined in section 6.1 above). If parameter recovery is successful, the model can then be run on the data from the 16study participants, to investigate whether there are any learning differences between positive and negative stimuli.

## Analysis of Experimental Question 3-4

Once the chosen model has been developed to include a parameter for positive and negative stimuli, we can then investigate whether the valence of the stimuli has an effect on learning rate, and to what extent this relationship is affected by anxiety.

Data will first be checked for normality using Anderson-Darling tests (with the null hypothesis of normally-distributed data being rejected at an alpha value of *p* < 0.05). Depending on the outcome, either a two-tailed paired t-test (for normally distributed data) or a Wilcoxon rank sum test (for non-normally distributed data) will be used to compare the learning rates for positive and negative stimuli trials. Results will be taken as significant if they meet the significance threshold of *p* < 0.05.

The next step will be to investigate whether anxiety levels have an effect on the difference in learning rates for positive and negative stimuli. First, we will calculate the difference in learning rate between positive and negative stimuli for each participant. Then, we will correlate the participant’s learning rate differences between positive and negative stimuli against their trait and state anxiety scores (as measured by the STAI-T and STAI-S questionnaires) as well as their score on the MAIA questionnaire. The relationship will be considered statistically significant if the Pearson’s r-value meets the significance threshold of *p* < 0.05.

## Analysis of Experimental Question 5

The final experimental question of this project is how participants’ level of certainty in their responses is related to anxiety. To answer this question, we will first calculate each participant’s average level of certainty across their responses – the level of certainty for each response is defined as the absolute value of difference between the response and 0.5 (each response has a value between 0.0 to 1.0 with 0.0 and 1.0 representing absolute certainty in either of the two possible outcomes, and 0.5 representing maximum uncertainty). Next, a Pearson’s correlation will be used to compare the participants’ average level of certainty with their trait- and state-anxiety scores on the STAI-T and STAI-S questionnaires, as well as with the scores on the MAIA questionnaire. The relationship will be assessed for significance using a threshold of *p* < 0.05.

1. **POSSIBLE EXTENSIONS AND MODIFICATIONS**

A possible extension that may be considered is to add additional parameters to capture environmental volatility within the model. This would allow us to investigate whether changes in volatility affect interoceptive learning, and how this might be affected by anxiety. Previous research has indicated that individuals with high anxiety are less able to adapt their outcome predictions to changes in the volatility of the environment compared to individuals with low anxiety9. Therefore, this may be an interesting factor to investigate further. However, this additional analysis would be contingent on both the initial model performing acceptably, and successful parameter recovery after adding volatility as a parameter.

Any changes or extensions to the analysis steps laid out in this plan will be documented and time-stamped.

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11. **REVISIONS**

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| **Author(s):** Katja Brand | | |
| **Approved by:** Olivia K. Harrison | | |
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| **Revision:** | **Date effective:** | **Description of change:** |
| Version 2 | 28.02.2022 | Changed number of participants.  Updated to include additional correlations with the STAI-S and MAIA questionnaires.  Changed description of use of l-ratio test. |