Short title:	NF-ASD SAP

Statistical Analysis Plan for

BRAINTRAIN: rt-fMRI NF intervention study in ASD

Clinicaltrials.gov No:	[**NCT02440451**]	Version Number:	v. 2.0

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Based on protocol version [*V1.1 and 14.02.2017*]:

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Protocol version	Updated Sap version no.	Section number changed	Description and reason for change	Date changed

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1. INTRODUCTION

This statistical analysis plan provides guidelines for the final presentation and analysis for the rt-fMRI trial. This plan, along with all other documents relating to the analysis of this trial, will be stored in the Statistical Analysis Master File electronically and/or in hard signed copy formats.

2. BACKGROUND

2.1 RATIONALE AND RESEARCH QUESTION

This study aims to demonstrate that fMRI neurofeedback training improves the ability to identify emotional facial expressions (and overall social behaviour) in subjects with ASD.

The intervention setup provides structured presentation of emotional facial expressions and the associated tools for mental imagery. We hypothesize that the accomplishment of the proposed competence training improves the subject's ability to comprehend facial expressions, identify emotions and be able to correctly express them.

To evaluate the improvements, we will use the results of the Facial Expression of Emotion: Stimuli and Tests (FEEST) - The Emotion Hexagon test. We expect that ASD subjects will be able to improve the number of expressions correctly recognized.

2.2 OBJECTIVES

The primary goal is to ensure improving the appropriate identification of emotional facial expressions, as measured by the Facial Expression of Emotion: Stimuli and Tests (FEEST) - The Emotion Hexagon test.

The secondary objectives are improvements in general aspects of social cognition derived from a generalization of the skills learned.

3. STUDY MATERIALS

3.1 TRIAL DESIGN

A Prospective, Single Arm, Longitudinal Cohort Study to assess improvement social attention in ASD (real –time fMRI neurofeedback approach).

The eligible patients for the study will be subject to the same experience and will be evaluated before (session 1) and after neurofeedback intervention (session 5 and follow-up).

3.2 RANDOMISATION

Not applicable.

3.3 SAMPLE SIZE

To determine de sample size, we used the G*Power tool (Faul, Erdfelder, Lang & Buchner, 2007):

Test family: t-test;

Statistical test: Means (difference between two different means);

Type of power analysis: A priori;

Fixed input parameters: Tail(s): Two;

α err prob: 0.05;

ES = 0.82*

Output: Estimated sample size

In these conditions, the estimated sample size is 14.

Without the normality assumption of the distribution of the means differences, we would also need 15 subjects, considering a non-parametric test.

Assuming a 20% dropout rate (based on literature) the sample size will be 18.

* (In the age group 20–30 Mean is known to be 109.00 (±SD=8.75) (out of 120). A previous study by Philip et al. (2010: Philip, RCM, Whalley, H, Stanfield, AC, Sprengelmeyer, R, Santos, IM, Young, AW, Atkinson, AP, Calder, AJ, Johnstone, EC, Lawrie, SM & Hall, J 2010) showed that in ASD deficits are present in identifying 'anger', 'sadness' and 'fear'.

In this study, the control group showed mean accuracy of 92.43% (±SD=7.57). The autism spectrum disorder (ASD) group was subdivided according to the Autism Diagnostic Observational Schedule (ADOS) score. The mean accuracy in the ADOS negative group was 81.33% (±SD 13.53) and in the ADOS positive group 76.45% (±SD 14.00)).

3.4 FRAMEWORK

In this study, we aim to determine if the training/modulation of the networks that integrate this region may influence an improvement/enhances the social behaviour/facial expression interpretation. Therefore, the main outcomes are testing for superiority rather.

3.5 INTERIM ANALYSES

No applicable.

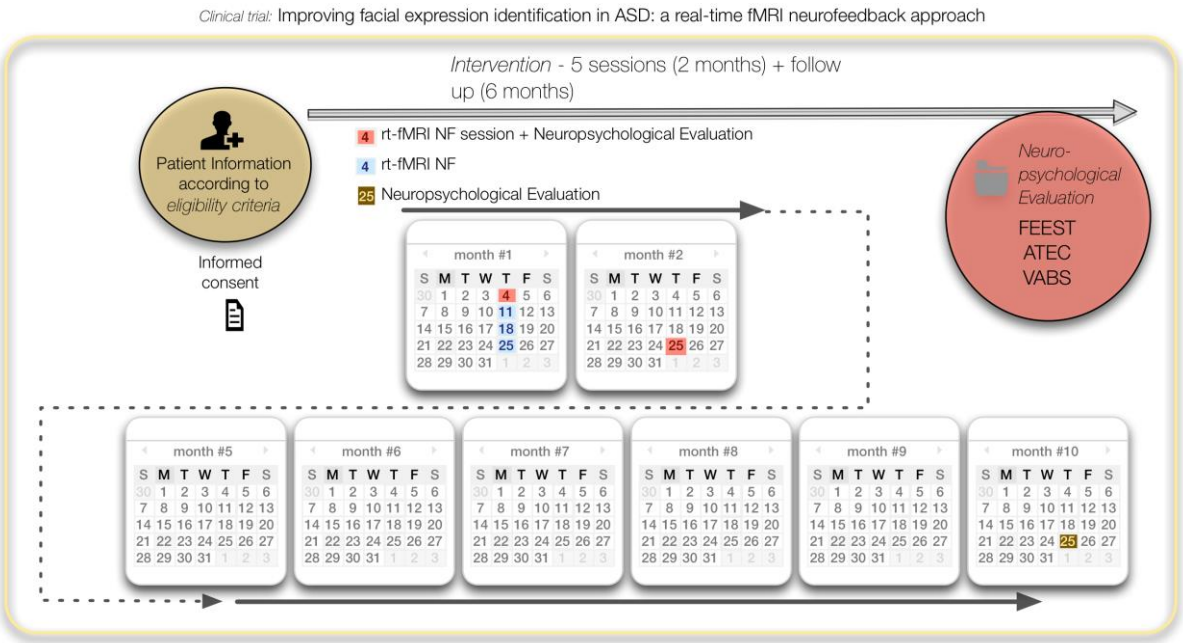
3.5.1 PLANNED SAMPLE SIZE ADJUSTMENT

3.5.2 STOPPING RULES

3.6 TIMING OF FINAL ANALYSIS

The intervention comprises five neurofeedback sessions spread over two months. Each subject will also undergo neuropsychological evolutions before the first session, after the last session (short-term) and in follow-up (long-term).

3.7 TIMING OF OUTCOME ASSESSMENT



4. STATISTICAL PRINCIPLES

4.1 LEVELS OF CONFIDENCE AND P-VALUES

All confidence intervals presented will be 95% and two-sided.

4.1.1 ADJUSTMENT FOR MULTIPLICITY

n/a

4.2 ADHERENCE AND PROTOCOL DEVIATIONS

4.2.1 DEFINITION AND ASSESSSMENT OF ADHERENCE

Adherence is defined as attending all five neurofeedback sessions.

Compliance is assessed based on the percent of subjects who have performed the scheduled number of interventional sessions. It is defined as:

% compliance = (number of sessions performed / number of planned sessions)*100%.

The number of planned sessions in this study is 5.

4.2.2 PRESENTATION OF ADHERENCE

Frequencies table and descriptive statistics of the adherence and “% compliance” will be summarized.

4.2.3 DEFINITION OF PROTOCOL DEVIATION

Physical incapacity to perform the tasks proposed.

4.2.4 PRESENTATION OF PROTOCOL DEVIATIONS

Register of number and type of protocol deviation and number of participants removed. No formal statistical testing will be undertaken.

4.3 ANALYSIS POPULATION

The intention-to-treat population will include all participants. If required the Centre for Trials Research will investigate methods of analysis that adjust for the number of sessions completed which is preferable to excluding participants which may result in biased results.

5. STUDY POPULATION

5.1 SCREENING DATA

Not applicable.

5.2 ELIGIBILITY

The number of participants eligible and how many were excluded due to violating each inclusion/exclusion criteria will be tabulated.

5.3 RECRUITMENT

A CONSORT flow diagram will be used to summarize the number of patients who were:

- assessed for eligibility
- lost to follow-up*
- discontinued the intervention*.

*reasons will be provided

5.4 WITHDRAWAL/FOLLOW UP

5.4.1 LEVEL OF WITHDRAWAL

The level of withdrawal will be tabulated, considering these aspects:

- Withdrawal from study intervention
- Withdrawal from study follow-up
- Withdrawal from entire study and does not want data to be used.

5.4.2 TIMING OF WITHDRAWAL

Timing of withdrawal from follow-up or lost to follow up data will be presented in a table. For each time point information on the number of withdrawals and reasons for withdrawal will be provided.

5.4.3 REASONS FOR WITHDRAWAL

A patient may withdraw or be withdrawn from the intervention for the following reasons:

- Withdrawal of consent for intervention by the participant
- Any alteration in the participants condition or circumstances which justifies the discontinuation of the intervention in the Investigators' opinion.

5.4.4 PRESENTATION OF WITHDRAWAL/LOSS TO FOLLOW-UP

The numbers (with reasons) of losses to follow-up (drop-outs and withdrawals) over the course of the study will be summarised in a table.

5.5 BASELINE PARTICPANT CHARACTERISTICS

5.5.1 LIST OF BASELINE DATA

Measure	Outcome(s)	Description
Demographic questionnaire	Age, gender, education, SES	
Thought control questionnaire (TCQ)(Wells & Davies, 1994)	Though control (distraction, punishment, worry; re-appraisal; social control)	30-item measure to assess effectiveness of strategies used for the control of unpleasant/unwanted thoughts
Thought control ability questionnaire (TCAQ)(Luciano et al., 2005)	Thought control	25-item measure of individual differences in perceived ability to control unwanted & intrusive thoughts
Wechsler Adult Intelligence Scale (WAIS-III)(Wechsler, 1997)	IQ	Global intelligence/IQ measure

5.5.2 DESCRIPTIVE STATISTICS

Categorical data will be summarised by numbers and percentages. Continuous data will be summarised by mean, standard deviation, minimum and maximum values. Tests of statistical significance will not be undertaken for baseline characteristics.

6. ANALYSIS

6.1 PROGRESSION CRITERIA

Since this study represents a feasibility trial this is not applicable.

6.2 OUTCOME DEFINITIONS

6.2.1 PRIMARY OUTCOME(S)

The results in the Facial Expression of Emotion: Stimuli and Tests (FEEST) - The Emotion Hexagon test will be the primary outcome measure. The group will be evaluated in the pre and post evaluation time with this test. The Emotion Hexagon test uses stimuli of graded difficulty, created using computer image manipulation techniques (morphing is used to modify photographs from the Ekman and Friesen (1976) series, creating examples that lie close to or more distant from the prototype expression). The 120 test trials with unambiguous stimuli (4 pictures for each of the 6 emotions across the 5 test blocks) can be used to derive an overall (total) score out of a possible maximum of 120 expressions correctly recognized.

6.2.2 TIMING, UNITS AND DERIVATION OF PRIMARY

The experimental group will be evaluated in the pre (session 1) and post intervention (session 5 and follow-up).

6.2.3 LIST OF SECONDARY OUTCOMES

The results in Autism Treatment Evaluation Checklist (ATEC) [Sociability and Cognitive Awareness Subtests] and the results in Vineland Adaptive Behaviour Scale (VABS) [Socialization and Daily Living Skills

Criteria	Level	Action
Consent rate	>50%	GO
	30-50%	Potential mitigating strategies
	<30%	STOP
Retention rate	>80%	GO
	50-80%	Potential mitigating strategies
	<50%	STOP
Intervention uptake: % of patients in the intervention group commencing the intervention	>90%	GO
	60-90%	Potential mitigating strategies
	<60%	STOP
Intervention adherence: % of attendance rates for patients in the intervention group	>80%	GO
	50%-80%	Potential mitigating strategies
	<50%	STOP
Acceptability of research processes and intervention assessed via qualitative interviews	Majority of reports positive	GO
	Minor issues identified that can potentially be resolved	Potential mitigating strategies
	Major problems with acceptability reported	STOP

Domains] will be one of the secondary outcome measures. The group will be evaluated in the pre and post evaluation time with this test.

6.2.4 ORDER OF TESTING

Not applicable.

6.2.5 TIMING, UNITS AND DERVICATION OF SECONDARYS

The experimental group will be evaluated in the pre (session 1) and post intervention (session 5 and follow-up).

6.3 ANALYSIS METHODS

6.3.1 LIST OF METHODS AND PRESENTATION

Initially will conduct an exploratory data analysis using graphical techniques (box and scatter plots) and quantitative analysis (statistical measures and frequency table) in order to characterize the sample, detect possible extreme outliers and measurement error.

To detect differences between the three time points of evaluation (session 1, session 5 and follow-up) will perform the Repeated-measures ANOVA. To identify the magnitude of the difference between each evaluation, 95% confidence intervals for the differences will be displayed in a table. (Note: considering a normal distribution)

6.3.2 COVARIATE ADJUSTMENT

Not applicable.

6.3.3 ASSUMPTION CHECKING

The Shapiro Wilks test will be performed to evaluate the normality.

6.3.4 ALTERNATIVE METHODS IF DISTRIBUTIONAL ASSUMPTIONS NOT MET

Without the normality assumption, we will consider a non-parametric tests: Friedman test and Wilcoxon or Sign test. Confidence intervals for medians will be presented rather than p-values where possible.

6.3.5 SENSITIVITY ANALYSES

Not applicable.

6.3.6 SUBGROUP ANALYSES

Not applicable.

6.4 MISSING DATA

Mean imputation, this is, the replacement of a missing observation with the mean of the non-missing observations for that variable.

6.5 ADDITIONAL ANALYSES

Not applicable.

6.6 HARMS

The number (and percentage) of patients experiencing each AE/SAE will be presented categorised by severity. For each patient, only the maximum severity experienced of each type of AE will be displayed. The number (and percentage) of occurrences of each AE/SAE will also be presented. No formal statistical testing will be undertaken.

6.7 STATISTICAL SOFTWARE

The analysis will be carried out using SPSS 23.0.

7. REFERENCES

7.1 NON STANDARD STATISTICAL METHODS

Not applicable.

7.2 DATA MANAGEMENT PLAN

This documents follows the instructions stated in the Data Management Plan (DMP) version 1.0.

7.3 TRIAL MASTER FILE AND STATISTICAL MASTER FILE

7.4 OTHER SOPS OR GUIDANCE DOCUMENTS

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SAP DEVIATION LOG

Document number:		**Document version:**	
Reason for deviation:			