IMPROVING FACIAL EXPRESSION IDENTIFICATION IN AUTISM SPECTRUM DISORDER (ASD): A REAL-TIME FMRI NEUROFEEDBACK APPROACH

PROTOCOL VERSION 14.02.2017

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Trial registration no:	**NCT02440451**

This protocol has been authorised by:			
Name	**Role**	**Signature**	**Date**
Miguel Castelo-Branco	PI for University of Coimbra		

General Information This protocol describes the **rt-fMRI NF intervention in ASD** study, and provides information about procedures for entering participants. The protocol should not be used as a guide, or as an aide-memoire for the care of other patients/participants. Every care has been taken in drafting this protocol; however, corrections or amendments may be necessary. These will be circulated to the local study team and the most current approved version will be available on the BRAINTRAIN extranet.

Compliance This study will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95). It will be conducted in compliance with the protocol and other regulatory requirements as appropriate.

The conducting of clinical trials on medicines for human use is governed by Law n.º 46/2004 of 19 August that implements Directive 2001/20/EC of the European Parliament and of the Council of 4 April in Portuguese legislation. In addition to the above legislation there are a series of guidelines covering various matters related to clinical trials which can be found in volume X of Eudralex (see link in PNEC portal below). To that end, and as part of the harmonised European system, the conducting of clinical trials in Portuguese research centres requires authorisation from INFARMED, I.P. (Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.) and a favourable, prior opinion from the CEIC (Clinical Research Ethics Committee).

We will register our trial to http://www.pnec.pt/portal/page/portal/PORTAL_PNEC. PNEC portal is a forum that allows all players to work together to transform research in Portugal, through a strategic approach that identifies national clinical research opportunities and barriers. All of the procedures described in this project have already been approved by the Ethics Committee of the Faculty of Medicine of the University of Coimbra.

The **rt-fMRI NF intervention study in ASD** study is funded by the European Commission 7th Framework Programme for Research, Technological Development and Demonstration, and is a component of Work Package 4.

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Clinical queries
Clinical Queries: Miguel Castelo-Branco (MD PhD) and Guiomar Oliveira (MD PhD)

Serious Adverse Events:

SAE reporting
Where the adverse event meets one of the serious categories, an SAE form should be completed within 24 hours of becoming aware of the event (See section 14 for more details).

Table of Contents

Glossary of abbreviations

AE	Adverse Event
ASD	Autism Spectrum Disorder
ATEC	Autism Treatment Evaluation Checklist
BDI	Beck Depression Inventory
CRF	Case Report Form
DMC	Data Monitoring Committee
EU	European Union
FEEST	Facial Expressions of Emotion – Stimuli and Tests
fMRI	Functional Magnetic Resonance Imaging
FP-7	7th Framework Programme for Research, Technological Development & Demonstration
GCP	Good Clinical Practice
HADS	Hospital Anxiety and Depression Scale
ICH	International Conference on Harmonization
ISRCTN	International Standard Randomised Controlled Trial Number
PIS	Patient Information Sheet
POMS	Profile of Mood States questionnaire
RCT	Randomised Controlled Trial
SAE	Serious Adverse Event
SEWTU	South East Wales Trials Unit
SOP	Standard Operating Procedure
STAIC	State/Trait Anxiety Inventory for Children
TCQ	Thought Control Questionnaire
TCAQ	Thought Control Ability Questionnaire
TMF	Trial Master File

TMG	Trial Management Group
TSC	Trial Steering Committee
USM	Urgent Safety Measure
VABS	Vineland Adaptive Behaviour Scales
WAIS-III	Wechsler Adult Intelligence Scale

1 Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
	V 1.0			
	V 1.2	14-02-2017	Susana Mouga, Bruno Direito, Bárbara Baía	*1.* Study Team: Bárbara Baía was included as the team *statistician.* 2. Figure presented in **Section 3.2 Participant flow diagram** was updated. Follow-up was included in the figure. 3. Text in **Section 4.2 Rationale for current study** was edited. Clarifications on the design and statistical plan were included. 4. Figure presented in **Section 6 Trial/study design** was updated. Follow-up was included in the figure. 5. Text in **Section 10.3 Intervention arms** was edited. Clarifications on the design and sessions schedule were included. 6. Text in **Section 12.5 Follow-up** was edited. Clarifications on the long-term analysis were included. 7. Text in **Section 13.2 Sample size** was edited. Clarifications on the statistical test were included. 8. Text in **Section 15.1 Main analysis** was edited. Clarifications on the statistical procedures that will be used in the main analysis were included.

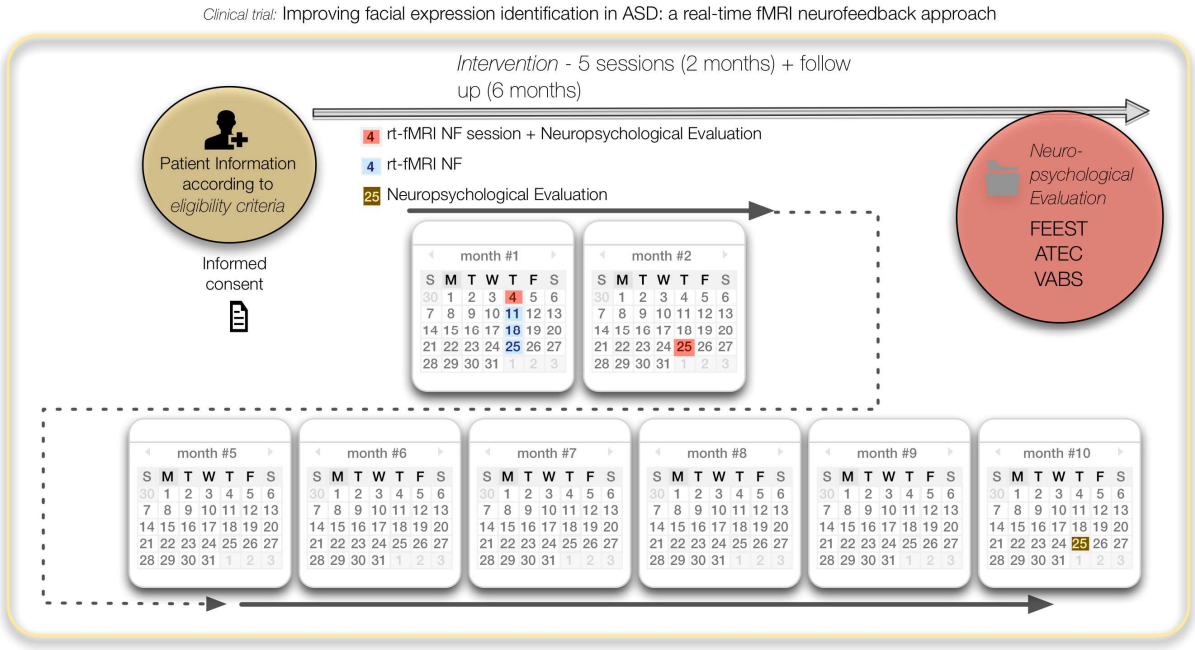
2 Synopsis

Short title	**rt-fMRI NF intervention study in ASD**
Acronym	NF-ASD
Internal ref. no.	IBILI – VB - 2015 – 02
Trial/study design	SINGLE ARM WITHIN – SUBJECT "BEFORE-AFTER" DESIGN.
Trial/study participants	ASD (experimental)
Planned sample size	15 (plus 3 in case of drop outs)
Follow-up duration	6 months after last session
Planned trial/study period	2 months (5 sessions, 4 sessions in first month and 1 one month later)
Primary objective	To improve social cognition in terms of facial emotional recognition
Secondary objectives	To generalize improvements to other social and cognitive domains.
Primary endpoint	Increase the score of successful emotion recognition in the Hexagon test
Secondary endpoints	Improve standard neuropsychological scores in clinical scales (Vineland, ATEC)
Interventions	rt-fMRI NF

3 Study summary & schema

3.1 Study schema

3.2 Participant flow diagram



3.3 Study summary

Clinical research has demonstrated that ASD children have deficits in the identification and interpretation of the emotional and mental state of others (Baron-Cohen 2001). An important skill to this end (impaired in ASD patients), is to appropriately recognize and discriminate emotional expressions.

The main brain regions involved in face processing are the inferior occipital gyri, lateral portion of the fusiform gyrus (especially a region deemed the fusiform face area or FFA), and posterior superior temporal sulcus (pSTS) (Haxby et al. 2000). Superior temporal sulcus (STS) plays a key role on several basic aspects in social information processing, and deficits in ASD have been found associated to this region.

The purpose of the study is to determine the effect of neurofeedback considering a social cognition brain region (i.e. pSTS) on the identification of facial expressions in ASD patients. The intervention comprises five neurofeedback sessions spread over two months. The first four sessions are weekly, and the last session performed one month later. In each session, the subjects are asked to imagine different facial expressions and their brain activity (i.e. BOLD activation in the pSTS region) is interpreted and used to estimate a feedback signal. We hypothesise that the training induces up-regulation of the target region (pSTS) that is highly related to clinical symptoms, as recognition of emotional states. This will improve

these specific clinical symptoms as can be measured by Facial Expression of Emotion: Stimuli and Tests (FEEST) - The Emotion Hexagon. Improvements the results in Autism Treatment Evaluation Checklist (ATEC) [Sociability and Cognitive Awareness Subtests] and in Vineland Adaptive Behaviour Scale (VABS) [Socialization and Daily Living Skills Domains] are also expected.

4 Introduction

4.1 Background

The technological advances raised from the use of multivariate supervised learning methods and from real-time functional magnetic resonance imaging (rt-fMRI) allows the development of new approaches capable of decoding a subject's brain state from functional magnetic resonance (fMRI) images (Formisano et al., 2008). The decoded brain states can be used as a control signal for a brain computer interface (BCI) or to provide neurofeedback (NF) to the subject (LaConte, 2011).

There is accumulating evidence from rtfMRI neurofeedback experiments that local brain activity measured by the blood oxygenation level dependent (BOLD) response can be specifically regulated (Weiskopf, 2012). Different brain regions have been identified as the target of NF studies, such as the somatosensory cortex (Bray et al., 2007; deCharms et al., 2004; Yoo et al., 2004), motor areas (LaConte et al., 2007; Sitaram et al., 2012; Weiskopf et al., 2004), amygdala (Johnston et al., 2010; Posse et al., 2003), anterior cingulate cortex (deCharms et al., 2005; Hamilton et al., 2011; Weiskopf et al., 2003) and visual areas (Scharnowski et al., 2012).

The brain activity associated with one particular state can be distinguished from alternate possibilities. The effectiveness of this identification is a key factor to determine whether the conscious and unconscious perceptual or cognitive states of an individual can be decoded. The ideal case of such separation is given when different cognitive states are encoded in spatially distinct locations of the brain resolved with the limited resolution of the fMRI (Haynes and Rees, 2006).

Emotional expression recognition

The identification of emotional and mental states of others is a major problem in individuals with ASD (Baron-Cohen, 2001). A fundamental skill to this process is the ability to recognize and discriminate emotional expressions. This skill is present from at least 10 weeks of age in typically developing infants (Haviland and Lelwica (1987) showed that 10 weeks old infants respond to three different facial expressions) and it continues to develop across childhood (Herba et al., 2006). ASD individuals show delays in the development of this ability (Baron-Cohen et al., 2001; Golan et al., 2008).

The main brain regions involved in face processing include the inferior occipital gyri, lateral portion of the fusiform gyrus (especially a region deemed the fusiform face area, or FFA), and pSTS (Haxby et al. 2000).

STS has been widely studied regarding social cognition processing and in particular the analysis of visual social cues (social perception) (Allison et al., 2000). Previous studies have concluded that STS plays a key role on several basic aspects in social information processing (biological movement, eye gaze) and furthermore, it was found that ASD subjects are affected by a decrease of gray matter in STS (Zilbovicius et al, 2013).

Some studies have identified differences in the facial processing of emotions in ASD subjects when compared with typically developing peers. Dapretto et al., 2006 found functional abnormalities in the frontal gyrus, which suggested a mirror neuron system deficit. It is common to find in the literature decreased activation in the fusiform gyrus in ASD individuals, when categorizing facial expressions of emotion (Hubl et al. 2003; Pierce et al. 2001; Schultz et al. 2000; Wang et al., 2004). However, it is not a universal finding (Bookheimer et al. 2008), especially when familiar faces are used as stimuli (Pierce and Redcay 2008).

Haxby and colleagues (2000) showed that the fusiform regions of the brain are more involved in processing constant characteristics of the faces, like identity, whereas the pSTS region is more involved in processing of dynamic characteristics, like eye gaze or facial expression.

A study from Ashwin and colleagues (2007) looked for responses to neutral, scrambled and fearful faces (with high and low intensity) and concluded that ASD individuals did not modulate FFA, medial pre-frontal cortex, amygdala, or STS in response to the different conditions, contrasting with the control group.

A NF study based on the up-regulation of emotional areas (including STS) was performed and showed that in the end participants were able to gain control over their brain activity. However, the training has been probably too short and longer NF sessions should be carried out (Johnston et al, 2011).

4.2 Rationale for current study

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.

Considering the prospective, single Arm, longitudinal design proposed, with alpha 0.05, power of 0.8 and standardized effect of 0.82 we would need 14 subjects. We will consider three additional subjects to account for drop-offs. The sample size calculations were performed under the assumption that the distribution of the mean differences is normal and based on a paired t-test (2-tailed). However, without the normality assumption, we would need 15 subjects, considering a non-parametric test.

To determine these values we used the G*Power tool (Faul, Erdfelder, Lang, & Buchner, 2007). Structure: (1) initial eligibility screening, (2) pre-intervention, (3) intervention process, (4) post-intervention, and (5) follow-up.

5 Study objective(s)

5.1 Primary objective(s)

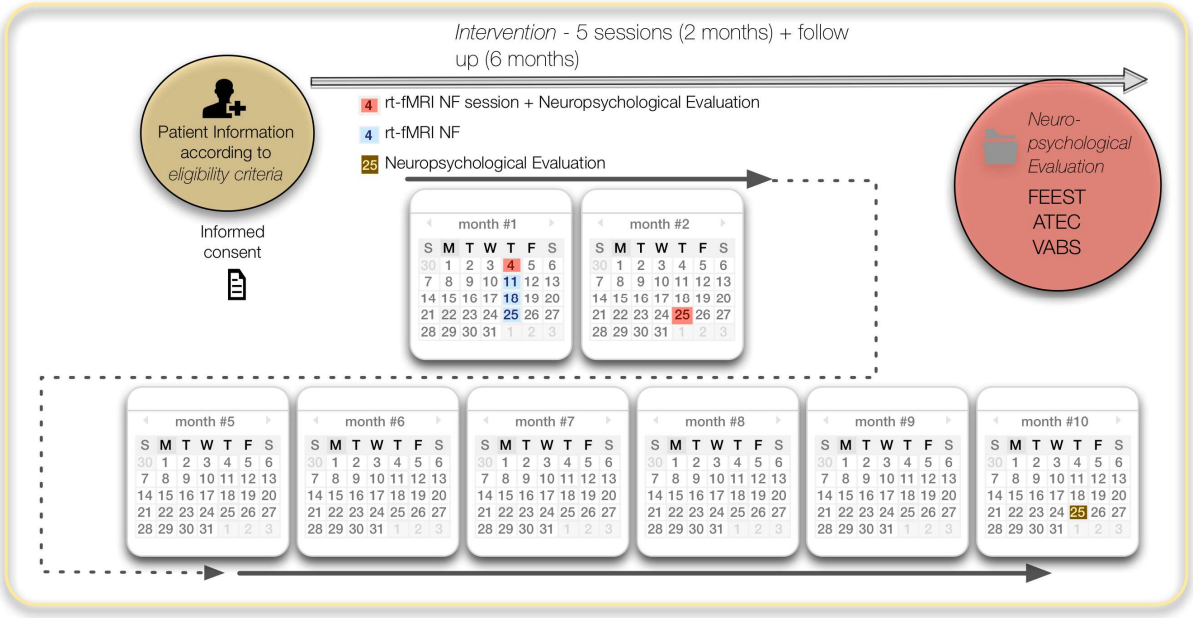
Improving the appropriate identification of emotional facial expressions, as measured by the Facial Expression of Emotion: Stimuli and Tests (FEEST) - The Emotion Hexagon test.

5.2 Secondary objectives

Generalization of learned skills to general aspects in social cognition.

6 Trial/study design

Clinical trial: Improving facial expression identification in ASD: a real-time fMRI neurofeedback approach



Allocation: not applicable

Endpoint Classification: Efficacy Study

Intervention Model: Single arm

Primary Purpose: Basic Science and clinical feasibility study

Participants: 15 ASD subjects (plus 3 in case of drop outs)

7 Participant selection

The eligible patients for the study should be high functioning ASD adolescents and adults. These participants should also meet all the following inclusion criteria and none of the exclusion criteria. All queries about patient eligibility should be directed to Susana Mouga before registration.

7.1 Inclusion criteria

- Positive diagnostic results for ASD in:
    - Autism Diagnostic Interview-Revised;
    - Autism Diagnostic Observation Schedule;

- The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (**DSM**-**5**) criteria.

7.2 Exclusion criteria

- Global Intelligence Quotient < 80
- Associated medical condition such as epilepsy, neurocutaneous or other genetic syndromes, or other usual comorbidity in ASD samples

8 Recruitment

8.1 Number of participants

A total of 15 (plus 3 in case of drop outs) participants will be recruited at a rate of two for each three weeks (see sample size calculation below).

8.2 Recruitment process

Participants will be recruited from the National Clinical Program for ASD at University of Coimbra. The Lead Researcher is responsible for contacting the participants.

8.3 Informed consent

Informed Consent form can be found attached in the appendices. This Informed Consent form must be signed by each participant/legal representative before the beginning of the study.

8.4 Screening logs

The data related to screening logs (paper format) will be kept in a secured room only accessible by BRAINTRAIN researchers. Additionally, data required to support analysis of the study will be entered and stored in an MS SQL Server database hosted on servers at Cardiff University. The hosting server is on a locked, secure machine room at Cardiff University.

The database will be backed up daily and access to the database will be via secure logon (username and password) and restricted to named study personnel only, as detailed in the study's PRA log. Each study will only see data and actions relating to their own study.

9 Withdrawal & loss to follow-up

Participants have the right to withdraw consent for participation in any aspect of the **rt-fMRI NF intervention in ASD** study at any time. The participant's care will not be affected at any time by declining to participate or withdrawing from the study.

If a participant initially consents but subsequently withdraws from the study, clear distinction must be made as to what aspect of the study the participant is withdrawing from. These aspects could be:

1. Withdrawal from study intervention

2. Withdrawal from study follow-up

3. Withdrawal from entire study and does not want data to be used.

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

In all instances participants who consent and subsequently withdraw should complete a withdrawal form or the withdrawal form should be completed on the participant's behalf by the researcher based on information provided by the participant.

10 Intervention

10.1 fMRI

During the scan radiographers will be able to see and monitor participants from the control room for the duration of the scan. Participants will be given a call button and can communicate through an intercom. The scan can be stopped at any time, should participants' request this or radiographers become concerned about a participant's health.

10.2 Possible undesirable effects

The known undesirable effects may include:

- The scanner may produce a loud noise when in use. Ear defenders or earplugs will be provided to help reduce this

- Some participants feel claustrophobic during the scan
- The scan is not painful but requires participants to remain motionless on a table for up to 45 minutes
- The scan may reveal incidental findings (see section 12.8)

10.3 Intervention arms

Experimental: Neurofeedback intervention

This group will undergo five sessions of neurofeedback intervention in the fMRI scanner. Each subject will also undergo neuropsychological evaluations before the first neurofeedback session, after the last neurofeedback session and on follow-up. The first four sessions are weekly while the last one is one month later. The intervention will take a total of two months.

10.4 Brain area/s of interest

Posterior Superior Temporal Sulcus

Rationale

pSTS has been associated with social perception processing/social conceptual knowledge (Moll et al., 2014; Saitovitch et al., 2012). 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.

10.5 Adherence

Adherence is defined as attending all five NF sessions.

After the enrolment, the participant will be given a schedule with the sessions plan. Additionally, the participant or caregiver will be reminded of each session by telephone-call one week earlier and by message the day before.

On the basis of following the study protocol, follow-up plan should depend on specific situation of participants such as avoiding the holiday or the inconvenient day. When the intervention is over, researchers should remind patients of the next appointment time. For eventual absent participants, researchers should do telephone interviews in time, ask the reason and try to reschedule.

11 Outcome measures

11.1 Primary outcome measure

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.

11.2 Secondary outcome measures

Secondary outcomes comprise the core measures detailed in Table 1 below.

- The results in Autism Treatment Evaluation Checklist (ATEC) [Sociability and Cognitive Awareness Subtests] will be one of the secondary outcome measures. The group will be evaluated in the pre and post evaluation time with this test.
- The results in Vineland Adaptive Behaviour Scale (VABS) [Socialization and Daily Living Skills Domains] will be one of the secondary outcome measures. The group will be evaluated in the pre and post evaluation time with this test.

Table 1. BRAINTRAIN core outcomes

Measure	**Outcome(s)**	**Description**	**Timepoint**
Demographic questionnaire	Age, gender, education, SES		Baseline
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	Baseline

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	Baseline
Wechsler Adult Intelligence Scale (WAIS-III)(Wechsler, 1997)	IQ	Global intelligence/IQ measure	Baseline
Profile of Mood States (POMS)(Heuchert and McNair, 2012)	Anger; confusion; depression; fatigue; tension and vigour		Pre-post assessment (each session)
Hospital Anxiety & Depression Scale (HADS)(Zigmond and Snaith, 1983)	Anxiety; depression	24-item scale (7 depression & 7 anxiety items)	Pre-post assessment (across intervention)
Beck Depression Inventory (BDI-II)(Beck et al., 1996)	Depression	21-item measure of clinical depression	Pre-post assessment (across intervention)

Autism Treatment Evaluation Checklist (ATEC) (Rimland and Edelson, 1999)	Autism Traits	77-items: speech/language/communication (14 items); sociability (20 items); sensory/cognitive awareness (18 items); health/physical/behaviour (25 items)	Pre-post assessment (across intervention)
Vineland Adaptive Behaviour Scales (VABS) (Sparrow et al., 1984)	Adaptive Behaviour	297 items measure of adaptive behaviour Three main domains: Communication (COM), Daily Living Skills (DLS) and Socialization (SOC) and total score, the Adaptive Behaviour Composite (ABC)	Pre-post assessment (across intervention)
Facial Expressions of Emotion – Stimuli and Tests - FEEST (The Emotion Hexagon test) (Young et al, 2002)	Emotion recognition	30 computer-manipulated images of faces (yielding a possible maximum of 120 expressions correctly recognized) from the Ekman and Friesen series to test recognition of basic emotions (anger, disgust, fear, happiness, sadness, and surprise)	Pre-post assessment (across intervention)
Debriefing interview questionnaire		Covers: strategies; general experience of the process; adverse effects;	Post-assessment (ideally each session)

12 Study procedures

12.1 Data collection

All data collected from the MRI scanner (training and testing steps) will be stored on the MRI setup. After each session, data will be copied onto a hard-drive for posterior analysis.

The tests related to the neuropsychological evaluation will be collected on paper at site and entered manually online into the information system.

12.2 Screening

Not applicable.

12.3 Pre-test

Participants will undergo an initial diagnostic and neuropsychological evaluation, followed by an *a priori* assessment of the outcome measures (i.e., Facial Expression of Emotion: Stimuli and Tests - FEEST: The Emotion Hexagon, Autism Treatment Evaluation Checklist (ATEC) [Sociability and Cognitive Awareness Subtests], Vineland Adaptive Behaviour Scale (VABS) [Socialization and Daily Living Skills Domains]), for establishing baseline metrics.

12.4 Post-test

Once concluded the intervention, the group perform the *a posteriori* evaluation, to assess the hypothesized improvements on the measures, as compared to pre-test assessment.

12.5 Follow-up

6 months for follow-up of neuropsychological performance (Hexagon, etc) and to assess the hypothesized improvements on the measures (long-term).

12.6 Samples & storage

Not applicable.

12.7 Facilities & equipment

fMRI scanning will be performed on a 3T Siemens Magnetom TimTrio scanner, at the Portuguese Brain Imaging Network, using a 12-channel head coil. For each participant, scanning will include the acquisition of blood oxygenation level-dependent (BOLD) contrast echo planar imaging (EPI) fMRI runs.

12.8 Incidental findings

At the time of consent, participants will be advised that scans will be performed using a research protocol and as such are different from diagnostic MRI and do not replace usual medical care. The scans may however reveal a suspected abnormality and the PIS and consent form should explain the possibility of incidental findings, and the procedure to be followed should this occur. Participants must explicitly consent to secondary review of their scans by a neurologist/radiologist should this be indicated, and to the research team contacting their doctor to advise further follow-up if necessary (or to direct referral for follow-up, depending on local procedures).

Should an MRI reveal a suspected abnormality, the following procedure will be followed:

- To avoid distress to participants arising from false alarms, staff must not disclose their concerns to the participant
- The authorised person must report their concerns to a neurologist/radiologist, supplying a digital copy of the image and a description of the cause for concern
- The neurologist/radiologist will review the scan and associated report and decide whether further follow-up is necessary
- In the event further follow-up is required, the Lead Researcher will be responsible for ensuring the participant's named doctor is contacted to arrange further follow-up.

13 Statistical considerations

13.1 Randomisation

Not Applicable

13.2 Sample size

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).

Assuming the later values, and conservatively expecting a normalization from 81.33% to 92.43% in a within subject design with a standardized effect size of 0.82, the required sample size at an alpha level of 0.05 and power of 0.8, would be 14 subjects. The sample size calculations were performed under the assumption that the distribution of the mean differences is normal and based on a paired t-test (2 tailed). However, without the normality assumption, we would need 15 subjects, considering a non-parametric test. To determine these values we used the G*Power tool (Faul, Erdfelder, Lang, & Buchner, 2007).

13.3 Missing, unused & spurious data

Detail provided in the Statistical Analysis Plan (SAP).

13.4 Procedures for reporting deviation(s) from the original SAP

These will be submitted as substantial amendments where applicable and recorded in subsequent versions of the protocol and SAP.

13.5 Termination of the study

Reasons for early termination of the study include unexpected unacceptable side effects.

14 Adverse Events

14.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a participant which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease.

Serious Adverse Event (SAE): Any adverse event that:

- Results in death

• Is life-threatening*

• Required hospitalisation or prolongation of existing hospitalisation**

• Results in persistent or significant disability or incapacity

• Consists of a congenital anomaly or birth defect

• Other medically important condition ***

* Note: The term "life-threatening" in the definition of serious refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** Note: Hospitalisation is defined as an inpatient admission, regardless of the length of stay, even if the hospitalisation is a precautionary measure, for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened or elective procedures does not constitute an adverse event.

*** Note: other events that may not result in death are not life-threatening, or do not require hospitalisation may be considered as a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

14.2 Causality

The assignment of causality should be made by the Investigator responsible for the care of the participant.

Table 2. Causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the study or intervention.
Unlikely	There is little evidence to suggest there is a casual relationship (e.g. the event did not occur within a reasonable time after intervention) with the study or intervention. There is another reasonable explanation for the event (e.g. the participant's clinical condition, other treatment).
Possible	There is some evidence to suggest a causal relationship with the study or intervention (e.g. because the event occurs within a reasonable time after intervention). However, the influence of other factors may

	have contributed to the event (e.g. the participant's clinical condition, other treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a judgement of the causal relationship.

14.3 Expectedness

The assessment of whether or not an SAE is an expected consequence of receiving the intervention will be provided by the Lead Researcher.

Depending on the nature of the event, the reporting procedures outlined in this protocol should be followed. Any queries concerning adverse event reporting should be directed to Miguel Castelo Branco - Chief investigator/Lead researcher.

14.4 Reporting procedures

14.4.1 Events exempt from reporting (non-serious AEs)

All such events, whether expected or not, should be recorded on the relevant case report form.

An SAE form should be completed for all SAEs within 24 hours. Events exempt from SAE reporting are set out in the Table below and information relating to them should instead be captured on the relevant CRF.

Table 2: Adverse Events exempt from reporting

Adverse Event	Information
Nauseas	Participants may suffer from nauseas during the fMRI acquisition.
Discomfort	Patients suffer from claustrophobia may suffer some discomfort due to the scanner dimensions.

Additional information should be sent within 5 days if the event has not resolved at the time of reporting. All events should be followed up through to resolution.

In the case of serious, unexpected and related adverse events, the main procedures to be followed are specified in the informed consent form. This document can be found attached in the appendices.

14.5 Urgent Safety Measures (USMs)

An urgent safety measure is an immediate change in a trial procedure or temporary halt to a trial procedure, put in place prior to approval in order to protect participants from any immediate hazard to health and safety following new safety information (SAE or other information received from an external source). The Lead Researcher may carry out USMs to protect participants from immediate harm.

15 Analysis

15.1 Main analysis

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 neuropsychological evaluation (session 1, session 5 and follow-up) will perform the Repeated-measures ANOVA. To identify the difference between each evaluation, the paired t tests will perform (note: considering a normal distribution).

However, without the normality assumption, we will consider a non-parametric test.

Further details will be found in the SAP.

15.1.1 Sub-group & interim analysis

Not applicable.

16 Data storage & retention

Data collected on paper will be kept in a secured room only accessible by BRAINTRAIN researchers, for a minimum period of 5 years after publication.

Data required to support analysis of the study will be entered and stored in an MS SQL Server database hosted on servers in a secure machine room at Cardiff University. This room is locked and entry is via a swipe card system.

The database will be backed up daily and access to the database will be via secure logon (username and password) and restricted to named study personnel only, as detailed in the study's PRA log.

17 Study closure

The trial will end up to 74 weeks after its start (including follow-up).

18 Regulatory issues

18.1 Ethical and research governance approval

The study will be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Ethical approval for this study will be given by the Ethical Committee of the Faculty of Medicine of University of Coimbra. Research governance approval will be granted by CEIC and will be sought after document approval.

18.2 Consent

Consent will only be considered informed following provision of adequate participant information (see attached information sheet and consent form). Participants will be informed that they are free to withdraw at any time and that this will not impact on current or future care.

18.3 Confidentiality

Data Confidentiality is assured as stated in the Informed Consent form, according with the Portuguese data protection regulations as per the EU directive. This document can be found attached in the appendices.

18.4 Indemnity

Although not expected due to participation in this study, if the subject suffers any injury as a result of any study procedures, performed according to the protocol, the subject will be reimbursed for medical

expenses necessary to treat them. All procedures are reviewed by staff and all incidents reported to appropriate persons and / or entities.

18.5 Trial sponsorship

Faculty of Medicine of University of Coimbra.

18.6 Funding

The Improving facial expression identification in ASD: a real-time fMRI neurofeedback approach study is funded by the European Commission 7th Framework Programme for Research, Technological Development and Demonstration.

18.7 Audits & inspections

The study may be participant to inspection and audit by CEIC /Infarmed.

19 Study/trial management

LBIM (Laboratory of Biostatistics and Medical Informatics of the Faculty of Medicine of University of Coimbra) will manage the trial.

We will keep, weekly project team meetings, monthly study management group meetings. These meetings will be attended by researchers directly involved in running the study.

20 Data monitoring & quality assurance

20.1 T/SSC (Trial/Study Steering Committee)

The TSC will be established centrally for the BRAINTRAIN consortium and include external clinical and methodological experts, at least one service user and an independent statistician. Members will be required to sign up to the remit and conditions as set out in the TSC Charter.

20.2 IDMC (Independent Data Monitoring Committee)

The nature of this study makes it unlikely that a separate Data Monitoring Committee *(*DMC) will be required; however, this will be discussed with the TSC at their first meeting and a DMC will be set up if deemed necessary. Members will be required to sign up to the remit and conditions as set out in the DMC Charter.

21 Publication policy

The Publication Policy is detailed in <<document title>> and can be found on the BRAINTRAIN extranet <<file location>>.

22 Milestones

Table 3. Milestones

TASK	START	DURATION	Months
			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Recruiting	1	5	
Intervention	2	12	
Follow-up	8	12	

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24 Appendices

- Informed Consent Form
- Ethical Committee Approval