STUDY PROTOCOL, STATISTICAL ANALYSIS PLAN AND INFORMED CONSENT FORM

Official Title

Multidimensional Study of Child and Parental Stress in Autism Spectrum Disorder After a Multisensory Intervention: Biomarkers, Sensory Responses and Clinical Assessment. A Randomised Controlled Trial

Item	Details
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Protocol version	Approved by the Research Ethics Committee of Universidad CEU Cardenal Herrera, report CEEI26/863 (25 June 2026)
Sponsor	Universidad Cardenal Herrera-CEU, Elche, Spain
Principal investigators	José Miguel Soria López, PhD; Ángela Sempere Pérez, MD, PhD
Study site	Centre de Teràpia Interfamiliar, Elche, Spain

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PART A. STUDY PROTOCOL

1. Administrative information

Item	Details
Official title	Multidimensional Study of Child and Parental Stress in Autism Spectrum Disorder After a Multisensory Intervention: Biomarkers, Sensory Responses and Clinical Assessment. A Randomised Controlled Trial
Title submitted to the ethics committee (Spanish)	Estudio Multidimensional del Estrés Infantil y parental en el Trastorno del Espectro Autista tras Intervención Multisensorial: Biomarcadores, Respuestas Sensoriales y Evaluación Clínica
Trial registration	ClinicalTrials.gov NCT number pending
Ethics approval	Favourable opinion of the Research Ethics Committee of Universidad CEU Cardenal Herrera, report CEEI26/863 (meeting of 25 June 2026; validity 5 years). Approved: clinical experimentation with humans, collection of human biological samples, and use of personal data through anonymous questionnaires.
Sponsor	Universidad Cardenal Herrera-CEU, Elche, Spain
Collaborating centre	Centre de Teràpia Interfamiliar (CTI), Elche, Spain
Principal investigators	José Miguel Soria López, PhD (Universidad Cardenal Herrera-CEU); Ángela Sempere Pérez, MD, PhD (Centre de Teràpia Interfamiliar)
Investigators	Universidad Cardenal Herrera-CEU: Sergio Montero Navarro, Nuria Javaloyes, Mª Ángeles García Esparza, Luis Eduardo Calderón Santos, Silvia Martínez Salazar. Centre de Teràpia Interfamiliar: Javier Sempere, Trinidad Pascual, Mª Carmen Moreno Blasco, Lara González Vázquez, Laura Paricio Martínez, Lidia Cebrián Jiménez, Marina Palazón, Esther Rufete.

2. Background and rationale

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised by persistent difficulties in social communication and interaction and by restricted, repetitive patterns of behaviour, often with sensory hyper- or hyporeactivity. Its estimated prevalence among 8-year-old children rose from about 1 in 150 in 2000 to about 1 in 31 in 2022.

Sensory processing differences are associated with anxiety, emotional dysregulation and poorer social and school adaptation. Children with ASD show heterogeneous activation of the hypothalamic-pituitary-adrenal (HPA) axis, including altered diurnal cortisol rhythms and atypical responses to social stressors. Elevated pro-inflammatory cytokines (IL-6, IL-1β, IL-17A) have been linked to symptom severity. Executive functions are also frequently affected and relate to adaptive functioning.

Parents of children with ASD experience high parenting stress because of the intensive care demands, with consequences for family wellbeing and quality of life.

Multisensory environments (MSE, Snoezelen®) offer structured, graded stimulation across visual, auditory, tactile, vestibular and proprioceptive channels to facilitate emotional and behavioural self-regulation. Their use is widespread, but evidence is limited by small samples, heterogeneous protocols, non-standardised outcomes and few controlled trials. This trial combines clinical scales, salivary and hair biomarkers (cortisol, oxytocin, interleukins) and autonomic recordings to evaluate an MSE programme against standard behavioural therapy.

3. Hypothesis and objectives

Hypothesis. Addressing stress physiology and sensory regulation in children with ASD through a multisensory room programme will improve sensory processing, self-regulation and stress in children, and reduce parenting stress, compared with standard behavioural therapy.

Primary objective. To evaluate the effect of a multisensory room intervention in children with ASD (support level 2-3) on stress, behaviour and attention in the children and on parenting stress in the main caregiver, compared with standard behavioural therapy.

Secondary objectives.

- To analyse changes in sensory processing after the multisensory intervention compared with standard behavioural therapy.
- To evaluate biological changes in stress-related biomarkers (salivary cortisol and oxytocin) and pro-inflammatory biomarkers (salivary IL-6, IL-1β, IL-17A and TNF-α).
- To examine changes in sustained attention, behavioural self-regulation and executive functioning compared with standard behavioural therapy.
- To analyse the relationship between sensory alterations, physiological stress and parenting stress in the main caregivers.

4. Trial design

Longitudinal, randomised, controlled, parallel-group trial with a pre-post intervention design and 1:1 allocation. The unit of randomisation is the family (child-caregiver dyad). Children in the experimental arm receive an 8-week structured multisensory room programme; children in the control arm receive standard behavioural therapy over the same period.

5. Study setting

Centre de Teràpia Interfamiliar (CTI), Elche (Alicante, Spain), in collaboration with the Neurobiology research group of Universidad CEU Cardenal Herrera (Elche campus), under an existing research collaboration agreement. Recruitment, interventions, sample collection and physiological recordings take place at the CTI; biomarker analyses are performed in the laboratories of Universidad CEU Cardenal Herrera.

6. Participants and eligibility

Children. Sixty children aged 3-12 years with a clinical diagnosis of ASD support level 2-3, diagnosed and registered at the CTI.

Inclusion criteria:

- Clinical diagnosis of ASD, support level 2-3.
- Age 3 to 12 years.
- Stable therapeutic and pharmacological treatment during the study period.
- Able to tolerate entering and remaining in the multisensory room.
- Informed consent from parents or legal guardians.

Exclusion criteria:

- Uncontrolled epilepsy.
- Recent changes in medication.
- Acute illness at the time of assessment.
- Severe motor or sensory disability preventing participation in the intervention.
- Persistent refusal of, or inability to adapt to, the multisensory room.

Caregivers. The main caregiver(s) of each enrolled child (n = 60).

- Inclusion: adult caregiver with sufficient cognitive capacity to understand the informed consent.
- Exclusion: acute illness at the time of assessment; severe motor or sensory disability preventing participation in the assessments.

7. Interventions

7.1 Multisensory room therapy (experimental arm)

A structured programme of one individual session per week for 8 weeks (8 sessions), each lasting about 45 minutes, delivered by occupational therapists with specific training in multisensory stimulation in ASD. Each session follows a semi-structured protocol:

Phase	Duration	Content
1. Welcome and initial adaptation	5 min	Gradual entry into the room with low stimulus intensity, dim light, no loud sounds and therapist accompaniment. The child's baseline state is recorded.
2. Guided sensory regulation	15-20 min	Graded stimuli adjusted to the child's sensory profile: soft visual stimuli, controlled auditory stimulation, tactile stimulation, gentle vestibular stimuli and proprioceptive stimuli. Intensity is increased progressively to avoid sensory overload.
3. Active participation and control of the environment	10-15 min	The child can choose or modulate some of the room's stimuli.
4. Closure and return to calm	5 min	Progressive reduction of stimuli, return to low lighting, predictable stimuli and structured closure.

The multisensory room includes visual (bubble columns, optical fibres, projectors, adjustable LED lights, unbreakable mirrors), auditory, tactile (texture panels, cushions, mats, sensory balls), olfactory (mild diffusers used with caution) and vestibular-proprioceptive elements (therapeutic swings, water bed, rockers).

7.2 Standard behavioural therapy (control arm)

Sessions of 45 minutes with psychology and speech therapy specifically addressing children with ASD support level 2-3, controlling time and interaction, under standard lighting, without therapeutic multisensory stimuli and with the same therapist presence as in the experimental arm.

7.3 Pre-session checklist

Before each session, families complete a checklist recording wake-up time, total sleep, breakfast, intense exercise or tooth brushing in the previous hour, medication taken, illness or fever in the previous 72 hours and other incidents, to control factors that may affect salivary biomarkers.

8. Outcomes

Primary outcome. Change in sensory processing measured with the Sensory Profile 2 (caregiver-completed), at baseline and after 8 weeks. Quadrants analysed: sensory seeking, avoiding, sensitivity (hyperreactivity) and registration (hyporeactivity).

Secondary outcomes.

- Executive function: BRIEF-P (preschool) and BRIEF-2, caregiver-rated (inhibitory control, flexibility, emotional regulation, initiation, working memory, planning/organisation, monitoring).
- Attention and behaviour: Conners-type scale (sustained attention, distractibility, impulsivity, behavioural regulation).
- Parenting stress: Parenting Stress Index-Short Form (PSI-SF).
- Child salivary biomarkers: cortisol, oxytocin and pro-inflammatory cytokines (IL-6, IL-1β, IL-17A, TNF-α), by ELISA.
- Caregiver biomarkers: salivary cortisol, oxytocin and cytokines; hair cortisol as an index of chronic stress.
- Caregiver autonomic recording (BIOPAC MP36R with AcqKnowledge 5): heart rate and heart rate variability (ECG, SS2LB leads), electrodermal activity (SS3LA), respiratory rate (SS5LB) and skin temperature (SS6L), over about 30 minutes.
- In-session self-regulation: structured observational record completed by the therapist after each session (8 items scored 0-3; total 0-24; higher = greater difficulty).

9. Schedule of assessments

Assessment	Population	T0 Baseline	T1 Each session (weeks 1-8)	T2 Week 8
Informed consent, eligibility	Caregiver / child	X		
Randomisation (1:1)	Dyad	X		
Pre-session family checklist	Caregiver		X	
Sensory Profile 2	Caregiver-reported	X		X
BRIEF-P / BRIEF-2	Caregiver-reported	X		X
Conners scale	Caregiver-reported	X		X
PSI-SF	Caregiver	X		X
Salivary cortisol, oxytocin, cytokines	Child	X	X (pre/post)	
Observational self-regulation record	Child (therapist-rated)		X (post)	
Salivary cortisol, oxytocin, cytokines	Caregiver	X		X
Hair cortisol	Caregiver	X		X
BIOPAC physiological recording	Caregiver	X		X
Adverse events	Child		X	X

10. Biological sampling and laboratory procedures

Saliva. About 1 mL of saliva is collected with a Salivette collector, before and after each therapy session in children and at baseline and week 8 in caregivers. Participants rinse their mouth with cold water, without brushing their teeth, and do not eat or drink in the hour before sampling. Samples are stored at 4 °C for up to 24 h, centrifuged at 2,500 rpm for 10 min, and the supernatant is stored at −20 °C until analysis. The protocol still lists three daily samples (08:00, 15:00, 23:00).

Hair (caregivers only). A hair sample of about 150 mg is cut from the scalp; the segment length reflects the period of interest (about 1 cm per month). Samples are pulverised, incubated in methanol for 36 h, and the supernatant is evaporated and reconstituted in phosphate-buffered saline.

Assays. Cortisol: Human Cortisol Competitive ELISA Kit (Invitrogen; sensitivity 0.024 ng/mL, range 0-30 ng/mL). Oxytocin: Oxytocin ELISA Kit (ab133050, Abcam). Cytokines: Human IL-6, IL-1β/IL-1F2, IL-17 and TNF-α Quantikine ELISA Kits. Plates are read on a Victor X5 reader.

11. Sample size

Sixty children (30 per arm) and their 60 main caregivers, estimated with G*Power allowing for 10% attrition and based on studies with similar objectives. Simple size was stimated G*Power for a two side comparison between independent group (α=0,05, power 0,80), assuming a large effect d=0,8. Based of a previous randomized trial of sensory based occupational therapy in authistic children (Schaff et al; 2014). This required 26 children per arm increase to 30 to allow for 10% attrition.

12. Recruitment, randomisation and blinding

Families of children with ASD support level 2-3 referred to and registered at the CTI who meet the eligibility criteria are invited by the CTI therapists, under the supervision of the principal investigators. After consent and baseline assessment, families are randomised 1:1 to the multisensory or control arm. Randomisation is stratified by age, sex and the primary outcome, in blocks, and the allocation sequence is concealed throughout the study.

Assessors are the same for the pre- and post-intervention measurements of each variable and are blinded to group allocation. Laboratory staff analyse coded samples. Therapists cannot be blinded; caregivers who complete the scales are aware of the allocation.

13. Data management and confidentiality

Data are recorded under a participant code without directly identifying information and stored on the servers of the CTI and Universidad CEU Cardenal Herrera, with access restricted to research team members by individual password. Only the research team with access to source data can link study data to clinical records. Processing complies with Regulation (EU) 2016/679, Spanish Organic Law 3/2018 (including the functional separation required by its Additional Provision 17.2.d) and Law 41/2002. Only coded data may be transferred, for the same purpose as the study; no international transfers are planned. Data are kept for at least 3 years after the end of the study.

14. Safety

Stimulus intensity in the multisensory room is adjusted progressively to the child's profile to avoid sensory overload, and sessions include a structured return to calm. Signs of stress or overload, behavioural crises and pauses are recorded at each session. A child may leave the study at any time at the request of the caregiver.

15. Ethics and dissemination

The study follows the Declaration of Helsinki, Good Clinical Practice and current legislation, and was approved by the Research Ethics Committee of Universidad CEU Cardenal Herrera (report CEEI26/863, 25 June 2026). All CTI investigators have signed a data confidentiality commitment (15 July 2026). All caregivers sign written informed consent for their own participation and for their child's participation before any study procedure. Families who do not sign the consent cannot take part. Results will be submitted to peer-reviewed journals and presented at scientific meetings.

PART B. STATISTICAL ANALYSIS PLAN

1. Software and general principles

- Analyses will be performed in R (R Foundation for Statistical Computing) and Jamovi by an analyst blinded to group allocation.
- Normality of quantitative variables will be assessed with the Kolmogorov-Smirnov test with Lilliefors correction and graphical methods.
- Descriptive statistics: n and percentage for categorical variables; mean and standard deviation, or median and interquartile range, for quantitative variables, depending on their distribution.
- All tests are two-sided with $\alpha = 0.05$. Effect estimates are reported with 95% confidence intervals.

2. Analysis populations

- **Intention-to-treat:** all randomised dyads, analysed in the arm to which they were allocated (primary analysis).
- **Per-protocol:** dyads in which the child attended at least 75% of the planned sessions (6 of 8) and completed the T2 assessment (sensitivity analysis).

3. Primary analysis

The Sensory Profile 2 score at week 8 will be compared between arms using analysis of covariance (ANCOVA), adjusting for the baseline score and the stratification variables (age group and sex). The adjusted mean difference between arms and its 95% CI will be reported. If model assumptions are not met, a rank-based ANCOVA or a Mann-Whitney test on change scores will be used.

4. Secondary analyses

- **Caregiver-reported scales (BRIEF-P/BRIEF-2, Conners, PSI-SF):** ANCOVA on the week-8 score adjusted for baseline, age group and sex.
- **Caregiver biomarkers and BIOPAC variables:** ANCOVA on week-8 values adjusted for baseline. Skewed biomarker concentrations will be log-transformed.
- **Child session-level biomarkers (pre/post each session):** linear mixed-effects models with arm, session number, pre/post, and their interactions as fixed effects, a random intercept per child, and sampling time of day as a covariate. The arm × pre/post interaction estimates the differential acute effect of the session.
- **Observational self-regulation record:** linear mixed-effects model of the total score across sessions, with arm, session and arm × session as fixed effects and a random intercept per child.
- **Within-group change:** paired t test or Wilcoxon signed-rank test, as appropriate.
- **Associations:** Pearson or Spearman correlations between sensory processing scores, physiological stress measures and parenting stress (secondary objective 4).

5. Missing data

The extent and pattern of missing data will be described. If data are plausibly missing at random, multiple imputation by chained equations will be used for the primary analysis, with complete-case analysis as a sensitivity analysis. Mixed-effects models use all available observations.

6. Multiplicity

There is a single primary outcome. Secondary outcomes are considered exploratory and will be interpreted with caution; no formal adjustment for multiple comparisons is planned.

7. Interim analyses and safety

No interim analysis is planned. Adverse events (behavioural crises, sensory overload requiring interruption) will be summarised descriptively by arm.

PART C. PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT FORM

> *English translation of the combined information sheet and informed consent form for participating families (approved under CEEI26/863; original in Spanish). Personal identification numbers are blank fields only.*

INFORMATION AND CONSENT DOCUMENT FOR PARTICIPATION IN A RESEARCH PROJECT

Responsible investigators: Dr Ángela Sempere (Centre de Teràpia Interfamiliar); Dr José Miguel Soria (Universidad CEU Cardenal Herrera).

1. Introduction

We are writing to inform you about a research study in which you are invited to take part together with your child. The study has been approved by a Research Ethics Committee of Universidad CEU Cardenal Herrera, with approval code CEEI26/863.

Our intention is that you receive correct and sufficient information so that you can decide whether to accept your participation and that of your child in this study. Please read this sheet carefully and we will clarify any questions you may have. You may also consult anyone you consider appropriate.

2. Voluntary participation

We invite you to take part in the study together with your child because your child has a compatible clinical diagnosis and you meet the inclusion and exclusion criteria.

Your participation in this study is voluntary and you may decide NOT to take part. If you decide to take part, you may change your decision and withdraw both consents (yours and your child's) at any time, without this affecting your relationship with your therapy centre or causing any harm to your care.

3. Aim of the study

The aim of this study is to evaluate the effect of a multisensory room intervention in children with autism spectrum disorder on the stress of parents and children, and on the children's behaviour, sensory alterations and attention, compared with a standard behavioural therapy.

4. Description of the study

The main aim of this project is to analyse, in a multidimensional way, stress in children with autism spectrum disorder and in their parents, and to evaluate the effects of an intervention based on multisensory therapy on this stress, as well as on the children's behaviour, sensory alterations and attention. The study will be carried out jointly by two entities: the Applied Neurobiology Research Group of Universidad CEU Cardenal Herrera and the Centre de Teràpia Interfamiliar, which allows clinical, academic and therapeutic perspectives to be integrated in a single research framework.

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised by persistent difficulties in communication and social interaction, together with restricted and repetitive behaviour patterns, frequently associated with alterations in sensory processing. These alterations, such as hyper- or hyporeactivity to stimuli, have been linked to higher levels of stress, anxiety and difficulties in the child's functional adaptation. Stress, understood as a physiological and psychological response to demands perceived as threatening, involves activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Although this response is adaptive under normal conditions, its sustained activation can lead to neuroendocrine alterations, such as chronic hypercortisolaemia, with a negative impact on emotional regulation and cognitive functions. Atypical stress-response patterns have been described in children with ASD, which increases their vulnerability to demanding environments. The impact of ASD also extends to the family: parents often experience high levels of parenting stress arising from the demands of care, which can negatively affect the quality of the emotional bond and patterns of interaction with the child. In this context, multisensory therapies (multisensory rooms) are a promising therapeutic tool.

These spaces provide structured and controlled sensory stimulation, promoting emotional self-regulation, reduction of disruptive behaviour and improved attention. However, evidence on their effects on objective stress biomarkers remains limited.

The project proposes an integrative methodology combining biological, physiological and psychological measures. Neuroendocrine biomarkers will be analysed by measuring cortisol and oxytocin in children and parents, and parental stress and the children's sensory alterations, behaviour and attention will be assessed with scales. In addition, the BIOPAC system will be used in parents to record physiological variables in real time, such as skin conductance, heart rate, heart rate variability, body temperature and respiratory rate, which are key indicators of sympathetic activity.

In short, this project aims to provide evidence on the role of multisensory interventions in modulating stress in ASD, as well as behaviour and attention. Its results may contribute to the development of preventive intervention programmes aimed at improving the wellbeing of the child and family, and at improving the learning and adaptation of children with ASD.

To compare the two therapies, the study is a randomised trial: participating families will be assigned at random (by lot, using a computer program) to one of two groups. In one group, your child will receive therapy in a multisensory room; in the other, your child will receive standard behavioural therapy with psychology and speech therapy, without multisensory stimulation. Neither you nor the research team will be able to choose the group, and you will have the same chance of being assigned to either one. In both groups, sessions will last about 45 minutes over 8 weeks, and the same assessments will be carried out.

The study has several parts:

If you accept the study and sign the informed consent, we will give you questionnaires to assess your stress, as well as your child's attention, sensory alterations and behaviour, before starting the intervention and at the end of it. At the same time points, physiological recordings will be taken from the parents with the BIOPAC system, together with saliva and hair samples to determine oxytocin and cortisol.

At each session, the children's behaviour will be assessed and saliva samples will be taken at the end of the session to determine oxytocin and cortisol.

The study will last 8 weeks and will take place at the Centre de Teràpia Interfamiliar with the support of CEU professionals.

The study does not involve any risk for the children and, if you agree to take part, you may withdraw at any time without giving explanations. In that case, the child's data would be removed from the computer files.

The investigators will ensure that confidentiality regarding the identification and data of the participants is maintained at all times. To this end, identification codes will be used without knowing the name of the person to whom the information belongs.

5. Personal data protection

In accordance with Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, repealing Directive 95/46/EC, you are informed that your personal data, obtained by completing this form and resulting from your participation in the project, will be processed under the responsibility of the CENTRE DE TERÀPIA INTERFAMILIAR and UNIVERSIDAD CEU CARDENAL HERRERA, for the purpose of managing your participation in this research project. You may contact the principal investigators, Dr Ángela Sempere (angela.sempere@uchceu.es) and José Miguel Soria (jose.soria@uchceu.es).

The data collected for the study will be identified by a code that does not include information that could identify you, and only the principal investigator of the study and collaborators will be able to link these data to you and to your clinical record. Therefore, neither your identity nor your child's will be disclosed to anyone, except in a medical emergency or where required by law. The processing, communication and transfer of personal data of all participants will comply with this law. Access to your identified personal information will be restricted to the principal investigator and collaborators, health authorities (Spanish Agency of Medicines and Medical Devices, foreign health authorities), the Research Ethics Committee and authorised personnel (study monitors, auditors), when they need to verify study data and procedures, always maintaining confidentiality in accordance with current legislation.

Data will be recorded in a research file under the responsibility of the institution and processed within the framework of your participation in this study. If the data from this study are used in future research related to the study or analysis, the sponsor will take the appropriate measures to guarantee the

protection of your privacy and will not allow your data to be cross-referenced with other databases that could allow your identification.

In accordance with data-protection legislation, you may exercise your rights of access, modification, objection and cancellation of data by contacting the principal investigator of the study. If you decide to withdraw your consent to take part in this study, no new data will be added to the database, but data already collected will be used.

6. Other relevant information

You and your child may be excluded from the study if the principal investigators consider it appropriate, for safety reasons, because of any adverse event, or because they consider that you are not complying with the established procedures. In any case, you will receive an adequate explanation of the reason for your withdrawal from the study. By signing this consent form, you undertake to comply with the study procedures that have been explained to you.

7. Contact in case of questions

If you have any questions during your participation or need more information, please contact Dr Ángela Sempere (angela.sempere@uchceu.es), principal investigator of this study.

8. SIGNATURE OF CONSENT

Mr/Ms _____, having read the preceding lines of this document, with identity document no. _____,

DECLARE:

That I give my consent to take part, together with my child, in the trial entitled “Multidimensional Study of Child Stress in Autism Spectrum Disorder after a Multisensory Intervention: Biomarkers, Sensory Responses and Clinical Assessment”. We have also been informed that our personal data and those of our child will be protected in accordance with Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 and Spanish Organic Law 3/2018 of 5 December on the Protection of Personal Data and Guarantee of Digital Rights, and will not be used without our prior consent.

Freely and voluntarily, I STATE THAT:

- ☐ I have read the information sheet I have been given about the study.
- ☐ I have been able to ask questions about the study.
- ☐ I have received sufficient information about the study.
- ☐ I understand that my participation is voluntary.
- ☐ I understand that I can withdraw from the study whenever I wish, without giving explanations and without this affecting the therapy received at the Centre de Teràpia Interfamiliar.

I will receive a signed and dated copy of this informed consent document, for which I freely give my agreement to participate in the study.

_____
The participant - signature, name and surname
Elche, on ____ of _____ 20____