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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Uncovering neuronal-based autism profiles using task-based fMRI and model-based clustering approaches

Mathilde Bironf

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

SUPERVISOR :
Prof. dr. Bert BRONE

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2022
2023

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www.uhasselt.be
Universiteit Hasselt
Campus Hasselt:
Martelarenlaan 42 | 3500 Hasselt
Campus Diepenbeek:
Agoralaan Gebouw D | 3590 Diepenbeek

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Uncovering neuronal-based autism profiles using task-based fMRI and model-based clustering approaches*.

Biront M.[1], Guendelman S.[1] and Dziobek I.[1]

[1] Clinical Psychology of Social Interaction, Department of Psychology, Humboldt Universität, Berlin, Germany

**FMRI clustering reveals ASD heterogeneity.*

To whom correspondence should be addressed: Prof Dr. Isabel Dziobek, Tel: (030) 2093-89758; Email: isabel.dziobek@hu-berlin.de

Keywords: Autism spectrum disorder (ASD); Social processes; Heterogeneity; Task-based neuroimaging, Unsupervised machine learning.

ABSTRACT

Traditional imaging approaches in mental health conditions assume homogeneity within clinical groups, obscuring the heterogeneity observed in autism spectrum disorder (ASD). This study aimed to identify clusters of individuals with ASD based on socio-emotional processes using the emotion regulation task (ERT) during functional magnetic resonance imaging. Blood-oxygen-level-dependent signals from predefined regions (inferior frontal gyrus and supplementary motor area) were utilized for unsupervised machine learning via Latent Profile Analysis. The study included 38 participants with ASD and 62 controls. Three clusters emerged: normative, low distinction, and high distinction, reflecting variations in brain activation across four different conditions during the ERT. The normative clustering showed no significant difference in brain activation compared to the control group. The low distinction cluster exhibits significant differences in brain activation in all conditions and behavioral responses during the regulation and non-regulation of positive emotions. The high distinction cluster showed significant differences in brain activation in all conditions and behavioral responses during not regulating positive emotions. No significant differences were found in age nor gender. Nonetheless, questionnaires showed significant differences among the clusters compared to the control group, except for the emotion regulation questionnaire, the social responsiveness scale, and the Duke Social Stress Scale. This study highlights the importance of individual variability to prove the heterogeneity. Additionally, these findings challenge traditional imaging approaches emphasizing the need to acknowledge heterogeneity in the clinical population. Further research is necessary to further validate the identified clusters, as understanding these diverse clusters within ASD may enhance personalized interventions for these individuals.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition diagnosed in 1% of the global population. ASD is a heterogeneous condition characterized by core features in two domains: 1) social communication, which is characterized by deficits in socio-emotional reciprocity, non-verbal communicative behaviors, and the ability to develop, maintain, and understand relationships, and 2) restricted, repetitive sensory-motor behaviors, which are characterized by stereotyped or repetitive motor movements of objects, ritualized patterns of (non-)verbal behavior, restricted, fixated interests that are abnormal in intensity or focus and hypo- or hyperreactivity to sensory input. These features are believed to result from early altered brain development and neural reorganization [1-7].

Despite extensive research, the etiology of ASD remains largely unknown. However, it is known that there is heterogeneity among individuals with ASD due to the impairments in different core regions of the brain [8]. These impairments implicate the behavioral manifestations observed in individuals with ASD. Impairments in these core regions, such as the

frontal lobe, superior temporal cortex, and amygdala, have been linked with deficits in social behavior. Other impairments, e.g. in the caudate nucleus and orbitofrontal cortex, have been linked to repetitive behavior [9, 10]. Furthermore, studies have proven that individuals with ASD often exhibit motor difficulties, linked to impairments in the motor cortex, including the supplementary motor cortex (SMA) and caudal motor cingulate area [11]. Neuroimaging studies have also revealed abnormal activation in the medial prefrontal cortex and various subcortical regions in individuals with ASD, which have been connected to cognitive and socio-emotional deficits. Notably, alterations in the frontal regions, such as the inferior frontal gyrus (IFG), including an enlargement in the gray matter, have been associated with language difficulties in ASD [12, 13]. Several studies have indicated that emotion regulation (ER) has been linked with the IFG and SMA. In individuals without ASD diagnosis, the IFG showed higher activation in ER using cognitive reappraisal, and SMA showed higher activation during tasks regarding ER [14, 15]. In ASD, individuals commonly encounter challenges related to ER [7].

ER refers to the capacity to purposefully modulate the intensity of affective reaction and their emotional state to facilitate adaptive functioning, representing a crucial adaptive response to changing environmental demands [3, 7]. ER encompasses an individual's ability to manage and modify emotional responses, such as duration and intensity, using behavioral strategies and cognitive processes to regulate continuous affective states and achieve personal goals. This skill is crucial for individuals who exhibit increased negative emotionality, characterized by a propensity for intense negative emotional responses to stimuli [7]. Proficiency in ER has been associated with increased positive social interactions and prosocial behaviors. Moreover, it is especially important for individuals who experience more significant negative affect, as negative emotionality is inversely related to prosocial behaviors and social competence [7]. ER skills rely on several prefrontal cortical areas involved in attention selection, working memory, and brain areas involved in processing emotional stimuli, including the amygdala or insula [14].

Impaired ER may serve as a potential explanatory framework for the observed deficits in individuals with ASD [3]. Furthermore, this impairment may also explain the increased prevalence of co-occurring conditions (e.g., attention deficit hyperactivity disorder or social anxiety disorder (SAD)) characterized by impaired ER in individuals with ASD [3, 16, 17]. Previous research has shown that individuals with ASD exhibit fewer increases in nucleus accumbens and amygdala activation during positive and negative emotional responses, respectively, resulting in a reduced change in dorsolateral prefrontal cortex activation compared to non-autistic individuals [3]. However, limited research has been conducted regarding difficulties with ER in adults with ASD and their potential treatment target in this population [7]. Thus, further research is needed to investigate deficits in ER among adults with ASD.

A feasible approach to investigate this is employing the research domain criteria (RDoC) framework. This RDoC represents a framework that focuses on a novel classification system for psychopathology. It is based on self-reports, observable behaviors, genetic traits, and neurobiological measurements [18]. The long-term goal of the RDoC framework is to achieve precision medicine in psychiatry by optimizing treatment outcomes for individual patients. The framework assumes that a diagnosis based solely on observable signs and symptoms cannot reflect heterogeneity in psychiatric disorders. The data from the RDoC fields of genetics and clinical neuroscience are valuable in identifying meaningful biomarkers which can be utilized for personalized treatment. However, the framework is now primarily valuable for explaining the neurobiology of psychiatric conditions such as ASD.

The RDoC matrix comprises six domains, including negative valence (e.g., anxiety, loss), positive valence (e.g., reward), cognitive systems (e.g., attention, working memory), social processes (e.g., affiliation), arousal/regulatory system (e.g., sleep-wake), and sensorimotor system (e.g., motor actions) [19-21]. These domains cover a range of processes involved in mental function, such as ER, cognitive control, and sensory-motor integration [21, 22]. The domains can be examined via molecules, genes, cells, circuits, physiology, behavior analysis, and self-reports. The six domains of the RDoC matrix are not intended to be comprehensive but rather represent a starting point for research into the biology of mental illness. The framework is currently only used to investigate emotions, post-traumatic stress disorder, and substance use disorder, but its potential application extends to

other mental illnesses, including ASD [19, 20].

This study focuses on investigating the differences in individuals with ASD according to the social processes domain of the RDoC, highlighting the existence of heterogeneity at the neural level, which could indicate the existence of clusters. Traditional imaging approaches, such as meta-analysis, pool limited effects across multiple studies and considers the most consistent on group average, which could obscure the possible difference on an individual level. The obscuring of heterogeneity within a population is still observed in imagining data in, e.g., the spatial location difference and signal's magnitude conceal different response patterns [23, 24]. To consider this issue, unsupervised machine learning (ML) techniques, specifically Latent Profile Analysis (LPA), were used to identify clusters of individuals with ASD who underwent an "emotion regulation task" (ERT) during functional magnetic resonance imaging (fMRI). Traditional regression-based models often address neural targets sequentially, ignoring heterogeneity and neglecting important multivariate patterns. This results in average effects that are less useful for informing diagnosis and treatment selection for individual patients [25]. In contrast, unsupervised ML can integrate multiple inputs across activated network components leading to personalized profiles that could inform treatment recommendations by predicting differential treatment responses to varying intervention approaches [23]. The resulting task-based fMRI data could offer a more comprehensive understanding of ASD heterogeneity and aid in personalized treatment selection for individuals with ASD [23, 25]. The ERT will be utilized as previous research has demonstrated deficits in ER among individuals with ASD [7]. The used ERT paradigm introduced a social regulation component. This social component contrasts with the commonly used ERT, which induces ER without including the social component. In the task used in this study, participants were presented with videos featuring individuals displaying specific facial expressions, such as smiling, to induce the social regulation component of their emotional responses.

Overall, our study aims to reveal the existence of clusters within ASD based on functional brain signals and compare these clusters with a brain-based profile derived from non-autistic controls. This will be done using unsupervised ML (LPA) to identify the brain-based cluster and comparison with the control group will be done via statistical analysis. We hypothesized that unsupervised ML applied to task-based fMRI data could identify multiple brain-based patient clusters. Additionally, we examined the potential relationship for validation of the identified clusters between brain-based patient clusters and the non-autistic controls based on self-reported behavioral responses during the ERT, age, gender, and self-reported questionnaires.

EXPERIMENTAL PROCEDURES

Participants – 100 participants, aged between 16 and 60, were recruited from the general population of Berlin and Freiburg. These participants group comprised participants diagnosed with ASD (N=38) and non-autistic control participants (N=62). Diagnosis of ASD was confirmed based on the Autism Diagnostic Observation Schedule (ADOS), conducted by researchers with formal training in conducting the ADOS [26]. Inclusion and exclusion criteria were applied to ensure homogeneity and appropriate representation in the study sample (Supplementary Table S1). All participants provided written informed consent prior to the investigation. The study was approved by the ethics committee of Humboldt-Universität, Berlin, and carried out according to the Declaration of Helsinki guidelines.

Self-questionnaires – Subjects completed online self-report questionnaires assessing socio-emotional functioning and regulation aspects. The questionnaires regarding social assessment included: 1) Autism Spectrum Quotient (AQ), which provides assessment traits associated with autism spectrum conditions, including 50 items using a 4-point Likert scale. 2) Social Responsiveness Scale (SRS), which assesses social responsiveness and impairment associated with ASD, including 65 items using a 5-point Likert scale. 3) Duke Social Support Stress Scale (DUSOCS), which measures perceived social support and stress, including 23 items using a 5-point Likert scale. 4) Social Anxiety Questionnaire (SASKO), which measures social anxiety symptoms, including 20 items using a 4-point Likert scale. The questionnaires for investigating emotional regulation and well-being included: 1) Beck Depression Inventory (BDI), which measures depressive symptoms, including 21 items using a 4-point Likert scale. 2) Difficulties in Emotional Regulation Scale (DERS), which assesses emotional regulation

difficulties, including 36 items using a 5-point Likert scale. 3) Emotion Regulation Questionnaire (ERQ), which assesses individual differences in ER strategies, including 10 items using a 7-point Likert scale. 4) Satisfaction with Life Scale (SWL-5), which measures global life satisfaction, including 5 items using a 7-point Likert scale. 5) Toronto Alexithymia Scale (TAS-20), which measures alexithymia, difficulty recognizing and describing emotions, including 20 items using a 5-point Likert scale. The Trier Inventory for Chronic Stress (TICS) was used to investigate the stress assessment. The TICS measures chronic stress, alexithymia, and difficulty recognizing and describing emotions, including 57 items using a 5-point Likert scale. These measures were chosen based on their established reliability and validity in assessing relevant socio-emotional functioning and regulation constructs.

RDoC domain – System for social processes

ERT activation – Subjects underwent the emotion regulation task (ERT) in an fMRI experiment. In the ERT, participants were asked to regulate their emotional reactions while watching a set of videos of actors displaying either positive or negative facial emotions for over 18 minutes. While watching these videos, the participants were instructed to regulate their emotional responses, with specific instruction to increase or decrease their emotions during the positive or negative videos respectively (resulting in two active regulation conditions, ER_pos = upregulating positive emotions, ER_neg = downregulating negative emotions). To establish a control condition, the same set of videos was presented to participants without explicit instruction to regulate their emotions. Participants watched the videos passively, without engaging in deliberate ER (resulting in two emotion reactivity conditions, Look_pos = positive emotion reactivity, Look_neg = negative emotion reactivity). After every set of videos for each condition, subjects self-reported their current emotional state with a Likert scale from 1 to 7 [14].

Scanning procedure – Brain imaging data were acquired using a Siemens 128-channel 3 Tesla MRI system (Siemens Healthineers, Berlin, Germany) with a 32-channel head coil at the Berlin Center for Advanced Neuroimaging (BCAN) in Berlin, Germany. Structural brain images were obtained with a Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence with a resolution of 1 x 1 x 1 mm^3 resolution, echo time (TE) of 2.22 ms, repetition time (TR) of 2500 ms, flip angle of 90° and a field of view of 100%. The acquisition matrix was set at 64 x 64 x 33 slices, with slice thickness, width, and depth of 0.80 mm. Slice acquisition was performed in a descending manner, resulting in a total of 1050 volumes acquired per subject. The functional brain images were obtained with the same sequence as the structural brain images, except for the TE and TR, the TE was 37.00 ms, and the TR was 800 ms.

Data preprocessing was done using *fMRI-prep* [27]. DICOM data was first arranged into BIDS format. Head motion correction was performed through rigid-body transformations using the MCFLIRT function before the time-domain filtering steps. Slices images were realigned to the middle of each TR with 3dTshift. Susceptibility-derived distortion correction using field map images was done with SDCFlows. Functional Echo-planar imaging images were registered to the high-resolution structural MPRAGE images using gray/white matter boundary-based registration (bbregister) and then transformed into standard space images (MNI_152) using nonlinear registration. Finally, non-aggressive ICA-AROMA was used to further denoising and control movement artifacts.

Preprocessed T2*-weighted BOLD images were used for statistical analysis. The processed images were entered into a general linear model (GLM) using *SPM software* version 12. At the individual level, a first-level analysis was performed, which involved incorporating regressors corresponding to each experimental condition. These conditions represented the 20 s intervals during which subjects were exposed to a video and regulated their emotions (4 conditions of interest: ER_pos, ER_neg_ Look_pos, Look_neg). These regressors were convolved with the canonical hemodynamic response function. For the second level of analysis, GLM-FLE with mixed effects modeling was employed, utilizing all first-level analyses as fixed effects. Contrasts of interest were formulated to investigate the effects of each experimental condition and the contrasts of ER_pos > Look_pos and ER_neg > Look_neg across the entire sample, including participants with ASD and control group. Whole-brain analyses were conducted separately for each condition (ER_pos, ER_neg_ Look_pos, Look_neg) and the specified contrasts. Statistic images were thresholded using a cluster-defining threshold of $p < 0.001$ and

corrected for multiple comparisons (FWE) using a cluster threshold of $p < 0.05$. Primary regions resulting from contrasts of ER_pos > Look_pos and ER_neg > Look_neg, derived from the whole sample, were subsequently selected as regions of interest (ROIs) for further analysis using ML analysis. Specifically, IFG and SMA were selected as the ROIs for subsequent analyses (Supplementary Table S2).

Analytic plan

To determine clusters of individuals with ASD using the selected ROIs, an unsupervised ML technique called Latent Profile Analyses (LPA), also known as Gaussian mixture modeling, was employed using *Mplus* software version 8.0 [28]. LPA was chosen due to its well-established capability to classify participants into qualitatively distinct subgroups or clusters based on the observed patterns of Blood-oxygen-level-dependent (BOLD) signals. It operates on a mixture modeling framework, employing categorical latent variables to explain observed response patterns based on multiple underlying distributions. This approach assumes that a set of variables can be adequately represented by a single distribution by its mean [23, 29].

Selection of Optimal cluster number – An iterative process was utilized to establish the appropriate number of patient clusters. Initially, a 1-cluster model was fitted to the data, followed by fitting a 2-cluster model and comparing it with the 1-cluster model. Subsequently, a 3-cluster model was fitted and compared with the 2-cluster model. This process continued by comparing each *k*-cluster model to a respective *k-1* cluster model up to a 6-cluster model. Model selection and evaluation were based on guidelines proposed by Maysn [30]. The determination of the final model was based on several factors, including: 1) Bayesian Information Criterion (BIC): A BIC of 10 between models indicates "very strong" evidence in favor of the model with the lowest BIC value. 2) Entropy: Entropy values ranging from 0 to 1 were considered, with values of 0.40, 0.60, and 0.80 representing low, medium, and high degrees of cluster separation, respectively.

Cluster validation – A comparison was conducted between the clusters based on socio-emotional functioning to validate the identified clusters. This comparison involved the assessment of behavior response using ERT and self-reported questionnaires using analysis of variance (ANOVA). Furthermore, demographic correlates, namely age, and gender, were analyzed using ANOVA for age and Chi-square test for gender associations.

Statistical analysis – Statistical analysis was conducted using SPSS software. Two-way analysis of variance (ANOVA) and Chi-square were employed if the data was continuous and categorical, respectively with Tukey HSD post hoc tests, after normality was checked with the Shapiro-Wilk test. Data were presented as mean and standard deviation (SD) with a significance level of 5%. Outliers were detected using the Grubbs test.

RESULTS

Optimal Cluster identification – A three cluster model was determined for the ASD population based on evaluating model fit indices in *Mplus* as the best fit for the data (Supplementary Fig. S1). Data for the clustering analysis was obtained from the BOLD-fMRI signals of the ROIs (IFG and SMA). The entropy value of 0.923, exceeding the threshold of 0.80 for model fit, indicated a high degree of cluster separation. The BIC value was 404.87 further supporting the selection of the 3-cluster model. While the 4-cluster model showed a higher entropy (0.938) and a lower BIC value (401.98), only one patient was assigned to cluster 4, therefore limiting the quality of this clustering.

Brain activation patterns in brain-based clustering – Based on the analysis of the brain activation patterns in IFG and SMA during the four different conditions (ER_pos, ER_neg_ Look_pos, Look_neg) of the ERT, four distinct patterns were observed when comparing the control group with the three clusters (Table 1, Fig 1). In the control group and the normative cluster (18%), high activation was observed during the ER_pos and Look_pos videos in both the IFG and SMA. Notably, the highest activation was observed in the IFG. No significant differences in brain activation were seen between the normative cluster and the control group. However, the low distinction cluster (58%) exhibited significantly ($p < 0.05$) lower activation across all four conditions in both the IFG and SMA compared to the control group. The high distinction cluster (24%) showed the lowest overall activation pattern compared to the control group using two group ANOVA. These findings highlight the variability in brain activation patterns during ER among the different clusters, with low distinction and high distinction clusters showing different

Table 1 – Mean BOLD activation observed in control group and clusters.

Qualitative description		Clusters		
	Control group	Normative	Low Distinction	High Distinction
ER_neg				
IFG	0.42	0.45	0.29*	-0.38*
SMA	0.26	0.37	0.39*	-0.14*
ER_pos				
IFG	1.27	1.02	0.33*	-0.39*
SMA	0.96	0.95	0.33*	-0.26*
Look_neg				
IFG	0.58	0.82	0.23*	-0.28*
SMA	0.47	0.77	0.25*	-0.14*
Look_pos				
IFG	1.49	1.22	0.31*	-0.012*
SMA	1.12	1.023	0.26*	-0.064*

Values represent the mean BOLD signal for control group and each cluster concerning the different ROIs during ER_neg, ER_pos, Look_neg, and Look_pos in the ERT.

* = two groups ANOVA comparing each cluster with control group ($p < 0.05$). BOLD, Blood-oxygen-level-dependent; IFG, inferior frontal gyrus; SMA, supplementary motor area; ER_neg, downregulating negative emotions; ER_pos, upregulating positive emotions; Look_neg, negative emotion reactivity; Look_pos, positive emotion reactivity.

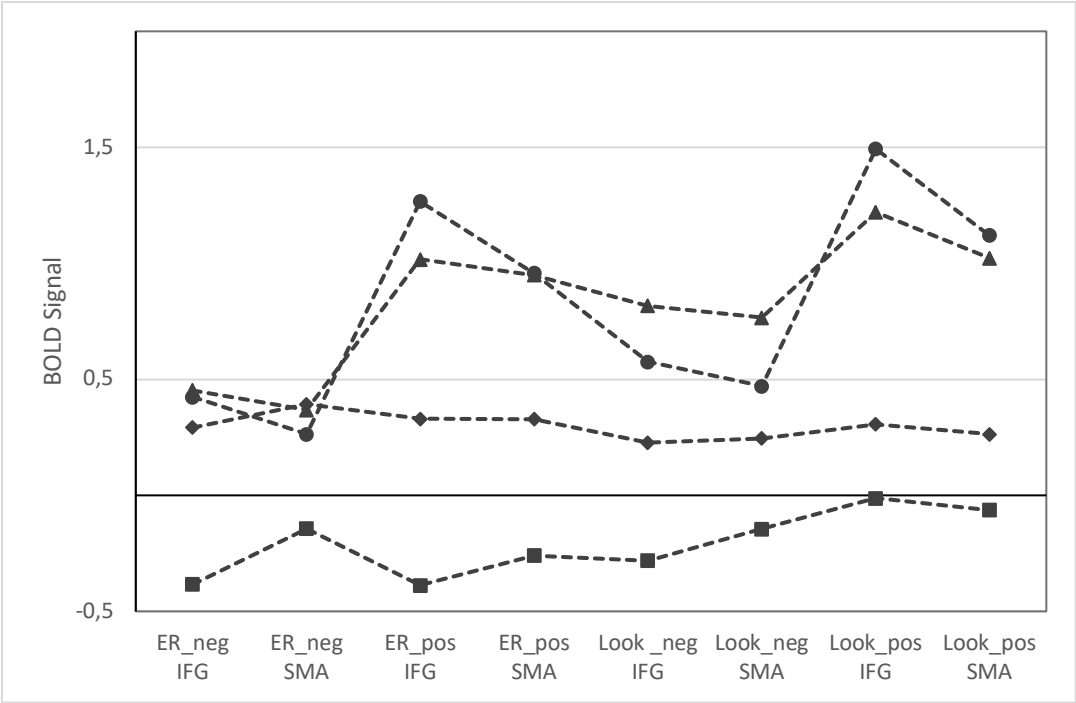

Fig. 1 – Brain activation patterns in IFG and SMA during ERT conditions. Brain activation patterns are observed among the control group (round), normative cluster (triangle), low distinction cluster (diamond), and high distinction cluster (square) during the four different conditions in both the IFG and SMA. High activation is observed in the IFG and SMA during ER_pos and Look_pos for the control and normative clusters. The low distinction cluster exhibits lower activation in both regions, while the high distinction cluster shows the lowest activation pattern compared to the control group. BOLD, Blood-oxygen-level-dependent; IFG, inferior frontal gyrus; SMA, supplementary motor area; ER_neg, downregulating negative emotions; ER_pos, upregulating positive emotions; Look_neg, negative emotion reactivity; Look_pos, positive emotion reactivity.

patterns of reduced activation compared to the control group.

Behavioral responses in brain-based clustering – Regarding the behavioral self-reported responses during ER_neg and look_neg in the ERT, no significant differences were observed with the two groups ANOVA when comparing the control group with each cluster. As to the behavioral self-reported responses during the positive condition (ER_pos and Look_pos) in the ERT, no significant differences were observed when comparing the normative cluster (M = 4.88, 4.08) with the control group (M = 5.24, 4.48). Additionally, no significant differences were found comparing the high distinction cluster in the ER_pos condition (M = 4.31) with the control group using the two groups ANOVA. However, a significant difference ($p < 0.05$) was seen using the two groups ANOVA between the control group and the low distinction cluster in both conditions (M = 3.82, 3.51), as well as in the high distinction cluster during Look_pos (M = 3.28), suggesting that individuals with ASD in the low and high distinction cluster had more difficulties regulating their positive emotions compared to the controls (Table 2).

Additionally, two-way group (normative, low distinction and high distinction cluster) by condition (ER_pos and Look_pos) ANOVA was used for the self-reported behavioral response, the main effect for condition was significant ($p < 0.05$), suggesting that in general, the participants with ASD can regulate their positive emotions. The main effect of the group was marginally significant ($p = 0.098$), this was mainly caused by the marginally significant ($p = 0.098$) difference between the normative cluster and the low distinction cluster. The group by condition interaction was not significant.

Two-way group (normative, low distinction and high distinction) by condition (ER_neg and Look_neg) ANOVA was used for the self-reported behavioral response, no significant difference was seen in the main effect and interaction effects.

Comparing Demographic and Questionnaire across brain-based Clusters – The demographic characteristics of age and gender did not show significant differences when comparing the control group with the three clusters separately (Table 3). In terms of the self-reported questionnaire, significant differences were found with the two groups ANOVA between control group and clusters in the social assessment measures (AQ, DUSOCS support, and SASKO) ($p < 0.05$). Regarding the AQ, there were significant differences observed between control group and the three clusters: the normative cluster (M = 7.22), the distinct cluster (M = 7.59), and the high distinction cluster (M = 8.29). Suggesting that in general, the participants with ASD have higher prevalence of autistic traits. Similarly, for the DUSCOS support, significant differences were found between control group and the normative cluster (M = 14.78), the low distinction cluster (M = 12.18), and the high distinction cluster (M = 14.00). Suggesting in general that participants with ASD perceive lower levels of social support. Lastly, the SASKO scores revealed significant differences between control group and the normative cluster (M = 71.78), the low distinction cluster (M = 70.64), and the high distinction cluster (M= 77.43). Suggesting in general that participants with ASD have a higher severity of social anxiety symptoms. However, no significant differences were observed in the DUSOCS stress and SRS questionnaires.

Regarding the questionnaires related to ER and well-being (BDI, DERS, SWL-5, and TAS-20), a significant difference was found between the control group and clusters. Significant differences were observed regarding the BDI between the control group and the

Table 2 – Self-reported behavioral responses during ERT observed in control group and clusters.

Qualitative description	**Clusters**			
	Control group	**Normative**	**Low Distinction**	**High Distinction**
ER_neg	2.05	2.02	2.34	2.64
ER_pos	5.24	4.88	3.82*	4.31
Look_neg	2.39	2.35	2.46	2.64
Look_pos	4.48	4.08	3.51*	3.28*

Values represent the mean scores for control group and each cluster concerning the behavior during negative and positive ER versus non-regulation in the ERT.

* = two groups ANOVA comparing each cluster with control group ($p<0.05$). ER_neg, downregulating negative emotions; ER_pos, upregulating positive emotions; Look_neg, negative emotion reactivity; Look_pos, positive emotion reactivity.

normative cluster (M = 11.11), the low distinction cluster (M = 13.55), and the high distinction cluster (M = 13.71). Suggesting in general that participants with ASD have a higher severity of depression symptoms. Similarly, for the DERS, significant differences were found between control group and the normative cluster (M = 48.56), the low distinction cluster (M = 53.77), and the high distinction cluster (M = 61.57). Suggesting in general that participants with ASD have a greater level of challenges in recognizing, accepting and effectively regulating emotions. Furthermore, for the SWL-5, significant differences were found between the control group and the normative cluster (M = 20.44), the low distinction cluster (M = 17.32), and the high distinction cluster (M = 14.86). Suggesting in general that participants with ASD have a reduced level of overall satisfaction of their life. Lastly, the TAS-20 revealed significant differences between control group and the normative cluster (M = 66.89), the low distinction cluster (M = 70.50), and the high distinction cluster (M = 75.57). Suggesting in general that participants

Table 3 – Demographic and questionnaire data observed in control group and clusters.

Variables		**Clusters**		
	Control group	**Normative**	**Low distinction**	**High distinction**
Demographic				
Age	33	30	37	34
Gender (F-M-NB) (%)	45-55-0	56-44-0	41-50-9	71-29-0
Questionnaires				
Social Assessment				
AQ	2.63 (1.71)	7.22* (1.86)	7.59* (2.48)	8.29* (2.36)
SRS	103.60 (17.63)	99.56 (25.66)	102.41 (28.25)	115.14 (27.25)
DUSOCS support	21.39 (7.05)	14.78* (6.67)	12.18* (6.30)	14.00* (10.94)
DUSOCS Stress	8.76 (4.29)	10.89 (6.05)	10.73 (4.98)	8.29 (8.36)
SASKO	27.27 (14.30)	71.78* (19.56)	70.64* (21.10)	77.43* (29.15)
Emotion Regulation & Well-being				
BDI	5.11 (4.97)	11.11* (8.58)	13.55* (11.49)	13.71* (13.65)
DERS	34.65 (9.51)	48.56* (8.44)	53.77* (11.62)	61.57* (13.24)
ERQ	39.1 (9.11)	38.33 (14.04)	38.77 (9.10)	34.29 (10.23)
SWL-5	26.47 (5.30)	20.44* (5.43)	17.32* (5.91)	14.86* (9.67)
TAS-20	35.97 (9.74)	66.89* (8.05)	70.50* (10.20)	75.57* (13.45)
Stress Assessment				
TICS	19.63 (5.17)	27.00* (5.68)	28.00* (7.22)	30.14* (11.54)

Values represent the mean (SD) for control group and each group concerning age, gender, and self-reported questionnaires.

* = two groups ANOVA and Chi-square comparing each cluster with control group ($p<0.05$). F, female; M, male; NB, non-binary; AQ, Autism Spectrum Quotient; SRS, Social Responsiveness Scale; DUSOCS, Duke Social Support Stress Scale; SASKO, Social Anxiety Questionnaire; BDI, Beck Depression Inventory; DERS, Difficulties in Emotional Regulation Scale; ERQ, Emotion Regulation Questionnaire; SWL-5, Satisfaction with Life Scale; TAS-20, Toronto Alexithymia Scale; TICS, Trier Inventory for Chronic Stress.

with ASD have a greater level of difficulty in identifying and expressing emotions. Only the ERQ questionnaire did not show significant differences.

When examining the stress assessment using the TICS questionnaire, significant differences were observed between the control group and the clusters, specifically between the control group and the normative cluster (M = 27.00), the low distinction cluster (M = 28.00), and the high distinction cluster (M = 30.14) Suggesting in general that participants with ASD have a greater level of perceived chronic stress. When comparing the three clusters with each other based on the socio-emotional functioning, no significant differences were found. However, a marginally significant difference ($p = 0.087$) was seen regarding the DERS, this was mainly caused by the marginally significant ($p = 0.071$) difference between the normative cluster and the high distinction cluster.

DISCUSSION

Our study revealed three brain-based clusters (normative cluster, low distinction cluster, and high distinction cluster) employing the LPA analysis within an ASD population, which indicates neural heterogeneity engaged in ER monitoring. Analysis of the brain activation pattern in the IFG and SMA revealed that the normative cluster exhibited activation patterns similar to the control group. In contrast, the low distinction and high distinction clusters demonstrated significantly lower activation in these regions. Regarding the self-reported behavioral responses during ERT, significant differences were seen between the control group and the low distinction in the ER_pos and Look_pos conditions. A significant difference was seen regarding the Look_pos when comparing the control group to the high distinction cluster. However, no significant differences were found between the control group and the clusters in the negative conditions (ER_neg and Look_neg) and when comparing the control group with the high distinction cluster for the ER_pos condition. Demographic comparisons, including age and gender, between the control group and the clusters showed no significant differences. Analysis of the self-reported questionnaires showed significant differences between the control group and the normative, low distinction, and high distinction clusters in various domains, including several questionnaires for social functioning, ER and well-being, and stress. However, no significant differences were observed in the SRS, ER, and DUSOCS stress questionnaires.

The three clusters determined based on these brain activation patterns were the normative clusters, which exhibited a brain activation pattern like that of the control group, as indicated by the absence of significant difference in IFG and SMA activation across all four experimental conditions (ER_pos, ER_neg, Look_pos and Look_neg). In contrast, low distinction and high distinction clusters displayed significantly lower activation in both the ROIs than the control group, with the high distinction cluster exhibiting the lowest brain activation pattern.

Our study's results could indicate potentially important differences in the IFG and SMA of individuals with ASD during ER. They suggest that each process in ER explores a different set of deviations from the controls. These findings are distinct from the commonly used analytical approaches that presume homogeneity within clinical groups. Such an assumption is prone to producing inconsistent findings and hindered replication when different combinations of patients are included in diverse samples.

A study investigating neural mechanisms in ER had previously reported lower activation in the nucleus accumbens and amygdala when individuals with ASD were instructed to increase positive and negative emotional responses [3]. However, the study only looked at the differences between the control and ASD population, not including the investigation of the heterogeneity seen within the ASD population. The low and high distinction cluster support these findings as these exhibited a lower activation pattern than the control groups in the ER_pos and Look_pos. However, no trend was seen comparing activation patterns during the negative emotion versus the positive emotion in the low and high distinction group, contrasting with the control group and the normative cluster. These findings could indicate differences between the individuals with ASD based on positive and negative emotions in the IFG and SMA.

The ERT behavioral self-reported responses analysis revealed differences between the control group and the identified clusters. Only a significant difference was observed between the low distinction cluster and control group regarding ER_pos and Look_pos and between the high distinction cluster and control group in the Look_pos conditions. When comparing the conditions (ER_pos and Look_pos), a significant

difference was seen, suggesting that, in general, the participants with ASD can regulate their positive emotions, which aligns with expected standards. A trend was seen between the normative cluster and low distinction cluster regarding the ER_pos and Look_pos. However, no trend was seen when investigating negative emotion conditions.

The results suggests that individuals with ASD during ER differ according to their behavioral responses when comparing the positive and negative conditions. This indicates that the clusters, as seen in the brain activation pattern, had different behavioral responses from the controls.

ER studies have proven that individuals with ASD have more difficulties using adaptive ER strategies. However, other studies report similar use of adaptive strategies for non-autistic participants [31]. Other studies have reported that individuals with ASD struggle to regulate their emotions positively [4, 32]. These results suggest that individuals from the low distinction and high distinction clusters have more difficulties upregulating their positive emotions, possibly due to inadequate management of emotions [32]. These findings could indicate heterogeneity within the ASD population, which is proven in the brain-based clustering analysis and contribute to the growing evidence of neural heterogeneity in ASD, accentuating the need to consider within-group variability when investigating individuals with ASD. However, more research needs to be done regarding ER in adults.

Interestingly, no significant differences were found between the clusters and control concerning age and gender. This lack of significant differences was also observed in the analysis of self-reported questionnaires SRS, DUSOCS stress, and ERQ. No trend was seen regarding age and DUSOCS stress. Interestingly, concerning gender, the high distinction cluster had a higher percentage of females (71%), which may reflect developmental factors. A recent study suggested that non-autistic females seek strategies for more social support, which could be more difficult for females with autism [31]. Additionally, the high distinction cluster had a higher mean in de SRS and a lower mean in the ERQ compared to the control group and the other clusters. However, because the cluster only existed out of 7 participants, no conclusion can be made for individuals with ASD. Significant differences were found in several questionnaires for social functioning, ER, and stress when comparing the control group with the three clusters separately.

These findings could not indicate the use of questionnaires for clustering an ASD population. As all clusters significantly different from the control group. Additionally, no significant difference between the clusters were seen. Noticeably, a trend was seen in the DERS between the normative cluster and high distinction cluster. Suggesting that individuals clustered in the high distinction group have more difficulties regarding ER, which supports the results seen in the data from the BOLD signals and from the self-reported behavioral responses. However, more research needs to be done the as the cluster only existed out of 7 participants. Other research already showed subgroups based on self-reported questionnaires, such as AQ and ERQ, indicating heterogeneity based on ER [33]. Therefore, we should not exclude the use of questionnaires for clustering.

More research should be done regarding differences within the cluster, as these were not significant based on the socio-emotional functioning. This lack of significance suggests that the brain-based clusters utilized in this study may not effectively capture the severity of psychopathology associated with ASD. Therefore, more investigation is needed to see if alternative measurements can be used for clustering an ASD population, as not much research has been done regarding the clustering of adult individuals with ASD based on ER.

Most research designs and analytic approaches (e.g., meta-analysis) and diagnostic processes assume homogeneity within the ASD population, focusing mainly on a group difference between the control group and the ASD population, which may overlook the diversity within ASD [31, 34]. This could lead to conflicting results and failure to replicate results, e.g., when another heterogeneous participant composition is included in different samples [23]. Our study addresses this limitation by examining brain-based clusters, revealing differences in neural activation patterns that may not be evident when treating ASD as a homogeneous entity. These neural findings are significant within our analytic approach, incorporating valuable information not commonly reported in classical unsupervised ML in mental health research. Specifically, we utilized a brain-based unsupervised ML approach, which offers several advantages compared to traditional k-means approaches. Our approach not only considers the

mean values but also estimates variance and provides information regarding the uncertainty in the degree of cluster separation and the effectiveness of each variable in differentiating the clusters [23].

By employing the RDoC framework to study the system of social processes, a comprehensive range of investigations encompassing brain activation patterns, self-reported behavioral responses, and questionnaires were conducted. This framework provides an understanding of ER in varying degrees of dysfunction. Our findings, comparing the three ways of analysis, suggest the existence of ASD clusters based on ER, as the three clusters showed differences compared to the controls at the level of brain activation, self-reported behavioral responses, and questionnaires. Interestingly, the brain-based clusters showed a stronger association with the ER_pos condition. This finding implies that the identified brain clusters may be more closely related to the positive emotion condition of ER, which is crucial for engaging in social interactions.

A limitation of this study was the use of only two ROIs (IFG and SMA) as these are known regions in the function of emotion cognition and regulation and came out of the contrast between conditions; ER_pos and Look_pos and ER_neg and Look_neg [15, 35-38]. No significant difference was seen in the validation between the clusters and compared to the control group when analyzing the ER_neg and Look_neg in the ERT behavioral self-reported response, age, gender, and some self-reported questionnaires. This could be due to the limited regions that have been investigated. Therefore, a more exploratory approach in brain regions that are known to be related to ER should be investigated, such as the complete ER network, including, for example, the amygdala, insula, or the dorsolateral prefrontal cortex, instead of only looking at the ROIs based on the actual contrast analysis [38, 39]. So additional brain regions could better display the heterogeneity found in the ASD population. Another limitation was that the small sample size, with only 38 out of 100 participants being diagnosed with ASD. This number is a relatively small sample size, as it is known that each subgroup should have at least 20 individuals, which should improve the power of the statistical analysis [40]. Therefore, more people should be investigated to have at least 20 individuals in each cluster.

The study only investigated individuals with ASD. However, it could be interesting to investigate other conditions within ER, such as SAD or depression, as it is known that these are the most prevalent affective disorder associated with ASD. These high rates of co-occurrence between these conditions could be due to ER deficits [3, 31]. Furthermore, conducting a longitudinal study would enable the investigation of whether ER could serve as a potential neural marker for treatment outcomes in individuals with ASD. Such a study could provide insights into the possibility of detecting treatment responses based on ER patterns over time.

Our study reveals the presence of clusters within the ASD population. These clusters showed different brain activation patterns indicating that the ASD population should not be evaluated as a homogeneous group. Diagnosis relies on behavioral criteria outlined in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental (DSM)-5, which has limitations regarding reliability, validity, and specificity for treatment selection. These limitations contribute to increased comorbidity, overdiagnosis, and insufficient guidance for clinicians. [41, 42]. Further research is necessary to improve diagnostics accuracy in ASD. The RDoC framework, focusing on social processes, shows promise in enhancing our understanding of the disorder. Therefore, the RDoC could help improve the diagnostic approach by gaining more information about ASD. This could lead to alternative methods to diagnose individuals with ASD, such as focusing on the brain activation pattern during emotional regulation. Despite advancements in various medical fields, the lack of understanding of the underlying mechanisms of ASD has hindered progress in diagnostic approaches and treatment. The heterogeneity and comorbidity within diagnostic categories and the high rates of co-occurrence among the disorders pose significant challenges to effective treatment and research in this field [43]. Therefore, our findings suggest that considering individual variability in neural activation and the behavioral pattern could be important for more personalized diagnosis and treatment of mental disorders, deviating from traditional categorical approaches that assume a homogenous clinical population.

CONCLUSION

This study represents an innovative investigation revealing brain-based clusters in an ASD population using unsupervised ML based on ER. Three distinct neural patterns associated with the

ASD population were identified and subsequently validated with the positive condition observed in the self-reported behavioral responses and multiple questionnaires, as the clusters exhibited significant differences from the controls. The unsupervised ML approach offers unique understanding beyond conventional autism phenotyping at the level of brain activation and socio-emotional function. The conventional phenotyping often captures only identifying a single phenotype despite multiple underlying physiological patterns contributing to ASD. This finding underscores the need for further research to analyze the heterogeneity within the ASD population and prioritize the examination of neurobehavior**.**

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Acknowledgments –The authors thank all the participants and students involved in the study. We also thank Dr. C. Kaufman and Dipl. -Psych T. Pinkman for all the technical support.

Author contributions –DI and GS conceived and designed the research. BM performed experiments and data analysis. KC aided with preprocessing the data analysis. BU aided in the fMRI scanning procedure. BM and GS wrote the paper. DI was accountable for all aspects of the work. All authors carefully edited the manuscript.

SUPPLEMENTAL

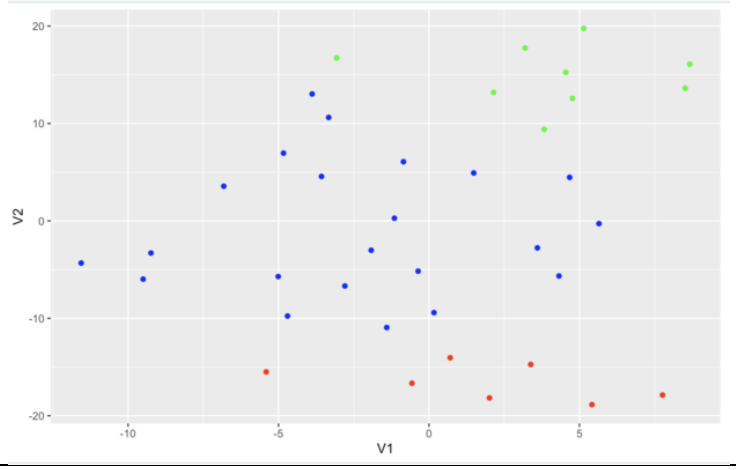
Supplemental Table S1. Including and excluding criteria for participants with autism spectrum disorder and the control group.

	Inclusion criteria	Exclusion criteria
Autism spectrum Disorder	Diagnosis of autism	Schizophrenia
		Bipolar disorder
		Suicidality
		Severe depression
		Severe disruptive behaviors not allowing group participation
Control group	N/A	Psychiatric disease(s)
		Taken psychotropic medication within the last three months
All	Age between 18-65	Known MRI contraindications such as metal implants, pacemakers, and claustrophobia
	IQ ≥ 80	
	Fluent German Language	

Supplemental Table S2. Coordinates of the regions of interest.

Region	Coordinates
Left IFG	-40, 28, -2
Right SMA	3, 17, 59

IFG, inferior frontal gyrus; SMA, supplementary motor area.



Supplemental Fig. S1 – T-SNE: Three different brain-based clusters during ER (ER_pos, ER_neg_ Look_pos, Look_neg). Three clusters were found via LPA analysis in *Mplus software*, the normative cluster (red), the low distinction cluster (blue), and the high distinction cluster (green). T-SNE table was made in the statistical *R studio software.*