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Specific dynamic facial expression evoked responses show distinct perceptual and attentional features in autism connected to social communication and GABA phenotypes

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Autism is characterised by core differences in social communication and interaction. The neurobiology underlying autism can be investigated using experimental designs that capture the dynamic nature of social perception, which activates the third visual pathway. Here, we investigated dynamic specific facial emotion processing using a naturalistic facial expression paradigm, leading to a specific dynamic N170 (dN170) evoked by emotion expression trajectories. Participants engaged in an active task of an avatar with two temporal trajectories: morphing from neutral to happy or sad expressions and unmorphing back to neutral. We recorded event-related potentials (ERPs) and magnetic resonance spectroscopy in autistic and non-autistic children and adolescents ($n = 16$ per group; ages between 8 and 17) matched for sex, handedness, and age. Results revealed that dN170 exhibited longer latencies during unmorphing for the autistic group. This specific timing effect, identified for the unmorphing versus morphing conditions in autism, suggests a stimulus trajectory-dependent effect (hysteresis). Dynamic P300 showed higher amplitudes in the autistic group during morphing, confirming the presence of an attentional compensatory mechanism. Correlations between ERP properties, GABA, and social communication abilities provided evidence of a dimensional continuum from non-autistic to autistic traits. These findings highlight the promising role of these ERPs as indicators of perceptual and attentional processing differences in autism.

Keywords Autism spectrum, Spectroscopy, Dynamic facial emotional processing, N170, P300, Children and adolescents

Autism is characterised by a distinctive engagement in social communication and interaction activities, such as socio-emotional reciprocity, understanding nonverbal cues (e.g., eye gaze, gestural communication, facial expressions of emotion) and initiating and/or maintaining social relationships[1]. These behavioural differences in the social cognition domain have been associated with atypical event related potentials (ERPs) during the processing of visual information[2–7]. Furthermore, the social communication differences observed in autism highlight the relevance of studying brain responses evoked by facial emotional recognition processes, which have

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an evolutionary significance recognised very early on by Darwin[8]. Emotion recognition relies on evolved brain regions and networks specialised in processing social and emotional cues, enabling adaptive responses in social settings[5]. These processes are key for interpreting facial expressions and regulating emotions and behaviours[5]. Moreover, ERP signals may provide evidence for neurobiological differences in neural processing in autism, given the high temporal resolution of electroencephalography (EEG) allowing the study of the chronometry of face processing[9,10].

ERPs evoked by a visual stimulus can be separated into early components (before ~200 ms) and late components (after ~200 ms). Early components, such as P100, have been associated with low-level stimulus features (e.g., contrast, luminance)[10,11]. One important early component is the face-sensitive N170[12,13]. It has a negative deflection peaking at 170 ms after presentation of a visual face stimulus[2,3] being linked to recognition and identification of configural information and evoked by emotional expressions[12,14]. This ERP can be recorded over the occipitotemporal scalp at the posterior region, originating within the fusiform gyrus[2,3] and the superior temporal sulcus (STS)[12,14]. Previous evidence shows longer latencies of the N170 component in autistic children, adolescents and adults suggesting a delay in face perception or social information processing, specifically for faces with emotional content[2,4,14,15]. Additionally, prior work shows smaller N170 amplitudes[14] during active tasks, and a left hemispheric bias with a relatively higher amplitude in autistic people during face processing[2,7,15]. Besides, a systematic review about facial expressions processing from childhood to adulthood supported neurophysiological differences in the N170 component between autistic and non-autistic individuals[16]. This review stresses the heterogeneity in behavioural performance between groups, with some findings suggesting that autistic participants may perform similarly to non-autistic individuals by employing compensatory mechanisms, reflecting a cognitive style rather than a deficit[16]. Additionally, a meta-analysis of visual information processing highlighted a distinct pattern of face processing in autistic individuals across the lifespan, showing improvements during neurodevelopment[2].

Concerning the late components, they reflect cognitive and socio-emotional processing of visual information representing high-level processing[2,10]. The P300 component represents a positive deflection at 300 ms over central/parietal areas and has been associated with top-down processing, including contextual evaluation[9]. It is modulated by emotional expressions[9]as well as attentional shifting towards a stimulus[15,17]. Furthermore, the P300 component has been studied using oddball paradigms based on the presentation of frequent stimuli and rare targets[18,19]. This paradigm focuses on identifying and responding to novel, unpredicted, and contextually unrelated information[19]. Therefore, it evokes a pronounced P300 component since the participant needs to rapidly detect and respond to the rare stimuli (target) activating more cognitive resources[18,19]. Previous studies have indicated longer P300 latencies in response to emotional expressions in autistic compared to non-autistic individuals[9]. In contrast, some studies have reported higher P300 amplitudes associated with greater activation in the precuneus during the decoding of facial expressions in autistic groups suggesting a more effortful attention-based compensation mechanism in the right hemisphere[6,17,20]. However, other P300 studies revealed inconsistent findings making it difficult to reach a unified conclusion about its role, due to task dependence, individual's age, stimulus type (e.g., verbal versus non-verbal) and the individual's required level of support[15,21]. Both N170 and P300 ERPs are related to socially relevant stimuli processing (e.g., eyes, faces, biological movement)[22]. Specifically, the N170 component was identified as an indicator of social functioning in autism[3] (i.e., slower N170 latencies to upright faces) emphasising the neurobiological differences in processing facial emotions[2,3,23,24]. These findings suggest a less efficient face processing or incomplete neurodevelopmental maturation[2,23] associated with social difficulties from childhood to adolescence[3]. This evidence suggests that the N170 (hereinafter referred to as "classical N170") latency is the most promising biomarker for autism across all ages[9,24,25]. Nonetheless, Aydin and collaborators highlight the absence of studies linking the P300 component to face processing in autism[9].

Social information processing (involving emotion processing and perspective taking) has been related to cortical inhibition and connectivity across social brain areas (e.g., posterior STS, temporoparietal junction, amygdala, and prefrontal cortex)[26]. Therefore, it is important to link neurophysiological patterns of activity with excitation/inhibition (E/I) imbalance, which has been suggested to be a contributing factor for social information processing differences in autism[26,27]. Our previous work in neurotypical participants further suggests that dynamic facial expressions evoked a dynamic N170 (dN170), which was found to be relate to social communication abilities and cortical GABA levels[28].

Magnetic Resonance Spectroscopy (MRS) allows the direct non-invasive in-vivo estimation and quantification of the main neurometabolites in the central nervous system, namely glutamate (contributing to "Glx", which corresponds to the sum of the overlapping of glutamate and glutamine resonances) and gamma-aminobutyric acid (GABA)[29–31]. These excitatory and inhibitory neurometabolites are pivotal in the E/I hypothesis[29,31,32]. However, GABA or GABA + measured in the occipital lobe have been reported to show no differences between non-autistic and autistic individuals[29]. Nevertheless, other studies have revealed a negative correlation between GABA + in the occipital region and an autism symptom severity measure (e.g[28,29]). Social communication and interaction scores measured by the Autism Diagnostic Interview-Revised (ADI-R)[33] communication subscale have been shown to be negatively correlated with GABA+, GABA+/tNAA and GABA+/tCR[27]. The relationship between GABA and Glx and behavioural profiles remain intriguing in autism (e.g[32,34]).

Glutathione (GSH) is an important antioxidant and neuromodulator[31] with various physiological functions (e.g., oxidation-reduction state balance)[30]. Alterations of brain GSH levels have been suggested as a potential pathophysiological indicator reflecting oxidative stress and inflammatory processes in autism[30]. However, there is relatively scarce evidence on the role of regional GSH and its impact on neurobiological differences in autism[30,31].

We previously demonstrated a relationship between GABA levels and social communication abilities and a novel dN170 component distinct from the classical N170[28]. We used a naturalistic paradigm with an active

task of an avatar morphing from neutral to happy or sad expressions and unmorphing back to neutral. Hence, we were able to elicit a pure dynamic expression face-sensitive dN170 isolated from the P100 component. This signal results from the pure contrast of a face expressing a dynamic emotion versus a face without an emotion. Then, a pure negative component, the dN170, and a dynamic P300 (dP300), both with delayed latencies, could be observed[28]. From a methodological perspective, we expected a delay on the first component, due to the time required to process motion information and interpret facial expressions, as the processing of dynamic emotional facial signatures is known to impact processing speed[6,28,35]. Besides, by using this paradigm we targeted the proposed third visual pathway, which is reportedly specialised in the dynamic features of face processing during social interaction[28,36]. This visual pathway is linked to early visual areas, motion-selective areas, and the STS, which supports dynamic face processing underlying socioemotional cognition[36]. EEG and functional magnetic resonance imaging studies have provided independent evidence for this distinct neurophysiological pathway, associated with high-level facial expression processing with a central node in the posterior STS[6,37,38]. Moreover, a more pronounced classical N170 component (i.e., with increased processing speed and higher amplitudes) was identified in the right hemisphere compared to the left, in both children and adults[10–13,16,28,39]. This right hemispheric lateralisation in social and facial emotional processing reflects an increased cortical specialisation in the right occipitotemporal cortex[10–13,16,28,39]. Nevertheless, in autism this hemispheric lateralisation had not been consistently found, with some studies reporting an absence[10,12] while others corroborate a right bias[6,16]. In our prior research, we also found evidence of a connection between difficulties in social communication abilities and longer dN170 latencies in the right hemisphere[28]. This is consistent with the findings showing a relationship between low GABA levels in visual face processing areas in the right extrastriate visual cortex (particularly at the middle occipital gyrus) and worse social communication abilities[28]. These results support the hypothesis of a spectrum of social communication competencies with a neurophysiological and neurochemical substrate in non-autistic children and adolescents. This highlights the forms of neurobiological diversity representing variations in brain development[28]. Here, we sought to investigate the ERPs components elicited during the above mentioned dynamic facial emotion recognition paradigm in autistic and non-autistic children and adolescents. Therefore, our goal is to deepen our understanding of the neurophysiological signatures evoked by dynamic conditions of temporal trajectory (morphing and unmorphing) between autistic and non-autistic individuals. Based on previous evidence, we formulated the following hypotheses:

Hypothesis 1 (H1): We expected longer dN170 latencies in the autistic group compared to the non-autistic group, as a function of the facial expression's temporal trajectory (group versus trajectory interaction).

Hypothesis 2 (H2): We expected to find longer latencies and higher amplitudes in the dP300 for the autistic group, depending on the facial expression's temporal trajectory (group versus trajectory interaction), reflecting a compensatory strategy in facial emotional processing, as reported in Simões et al.[6] study using a similar paradigm.

Hypothesis 3 (H3): Based on our previous work, we expected to find longer dN170 latencies associated with lower social communication abilities and GABA levels negatively associated with a measure of autism characteristics, the Social Communication Questionnaire (SCQ)[40] (i.e., lower GABA concentrations correlated with social communication difficulties). Therefore, we performed correlation analyses to investigate the complex interplay between neurophysiological signatures, neurometabolites profiles, and social communication abilities (i.e., behavioural profiles) in a continuum from non-autistic to autistic groups.

Finally, bearing in mind our three main hypotheses and on our previous studies[6,28,] we expected to find evidence of a right hemispheric bias.

Results

ERPs evoked by a dynamic facial emotion recognition task

As previously reported in Sousa et al. study in neurotypicals[28]the dynamic facial emotion recognition task (Fig. 1a and b) yielded a specific face expression sensitive dN170, resulting from the contrast between facial expressions and the neutral face baseline, which removes the P100 component, leaving only a face expression specific component.

Figure 2 shows the neurophysiological signatures specifically evoked by the dynamic facial expression trajectories, namely the morphing and unmorphing conditions, for both autistic and non-autistic groups.

Hypothesis 1 (H1): dN170 signature at the latency level.

Regarding the dN170 amplitude, a statistically significant two-way interaction between hemisphere and group was found, $F(1, 30) = 5.00$, $p = .033$, $\eta p^2 = 0.14$ (see Figure S3a). However, this result did not survive post-hoc comparisons. Moreover, a main effect of dynamic conditions was found, $F(1, 30) = 15.56$, $p < .001$, $\eta p^2 = 0.34$, revealing greater dN170 amplitudes in the morphing condition ($M = -2.70$, $SE = 0.24$) compared to the unmorphing condition ($M = -1.88$, $SE = 0.17$). In addition, a main effect of hemisphere was identified, $F(1, 30) = 12.17$, $p = .002$, $\eta p^2 = 0.29$, indicating a right hemisphere bias, amplitudes were greater in the right hemisphere ($M = -2.85$, $SE = 0.28$) compared to the left ($M = -1.73$, $SE = 0.20$), regardless of group or dynamic condition.

Concerning dN170 latency, a statistically significant two-way interaction was found between dynamic condition and group, $F(1, 30) = 5.38$, $p = .027$, $\eta p^2 = 0.15$ (see Fig. 3 and S3b). Post-hoc comparisons revealed a longer dN170 latency in the autistic group ($M = 263.81$, $SE = 8.89$) compared to the non-autistic group ($M = 235.06$,

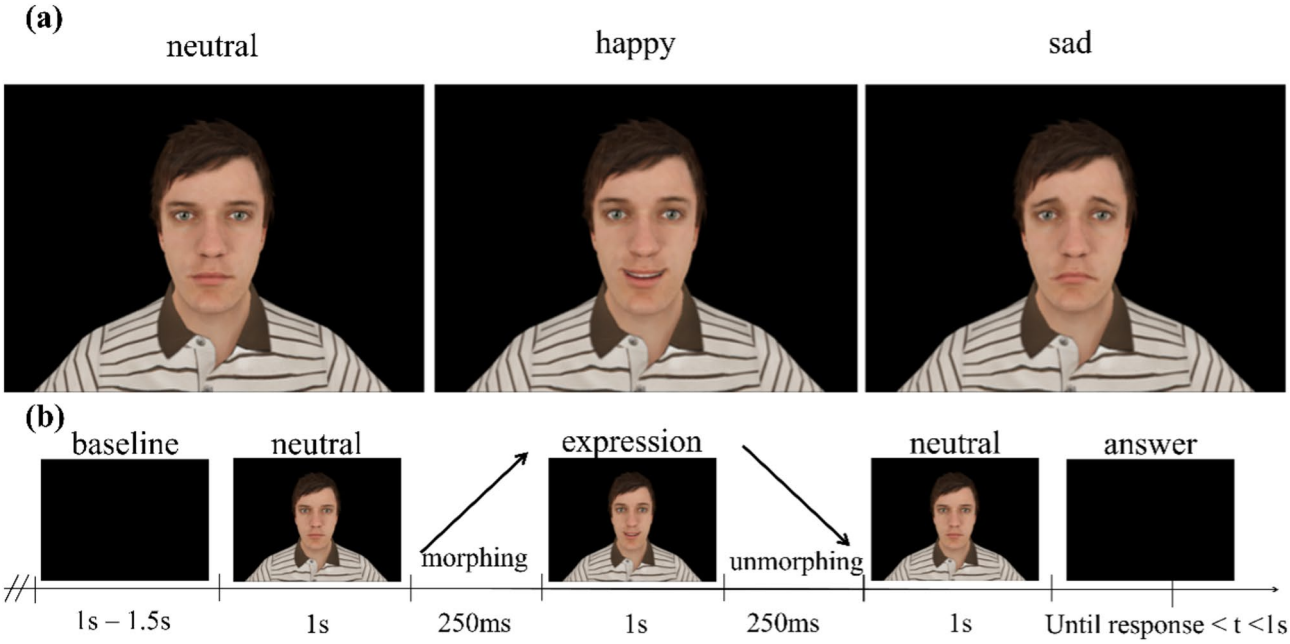

Fig. 1. (**a**) Facial expressions used as stimuli in the experiment. (**b**) Structure of the trials. Happy and sad facial expressions took 1.5 s, separated by facial expression morphing, static facial expression presentation and facial expression unmorphing. Previously published in Sousa et al.[28] with permission of Creative Commons Copyright.

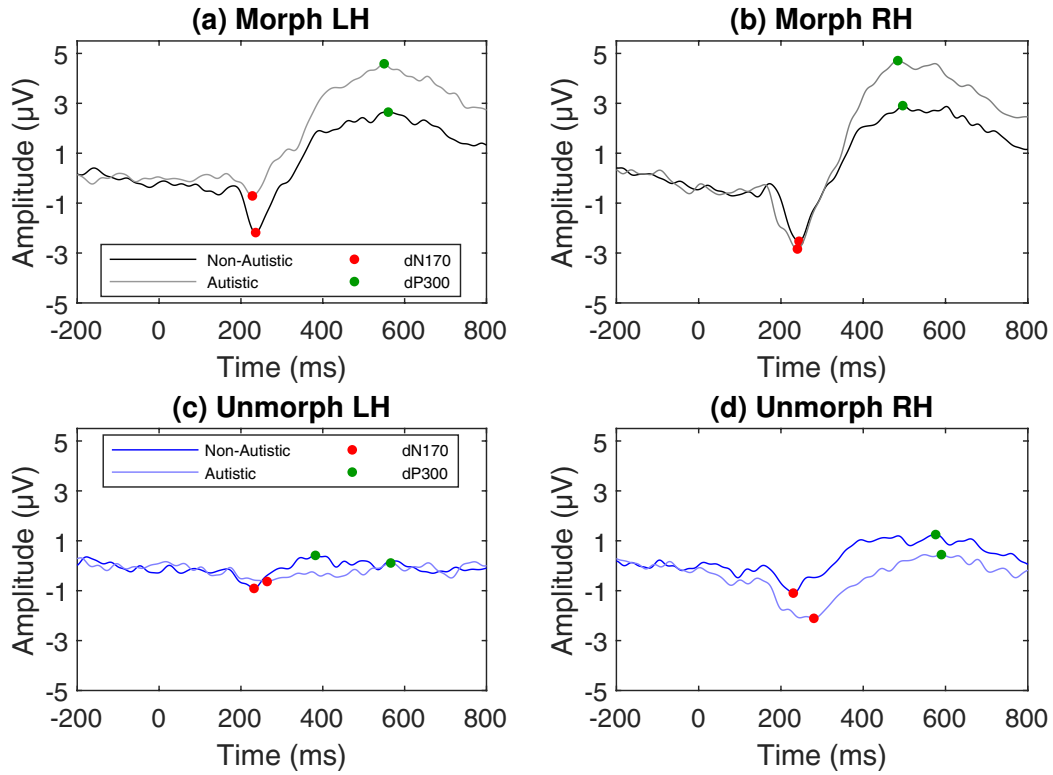

Fig. 2. ERPs evoked by (**a**) Morphing of a full expression in the left hemisphere (LH), (**b**) Morphing of full expression in the right hemisphere (RH), (**c**) Unmorphing from a full expression to neutral/static in the LH, and (**d**) Unmorphing from the full expression to neutral/static in RH for both groups.

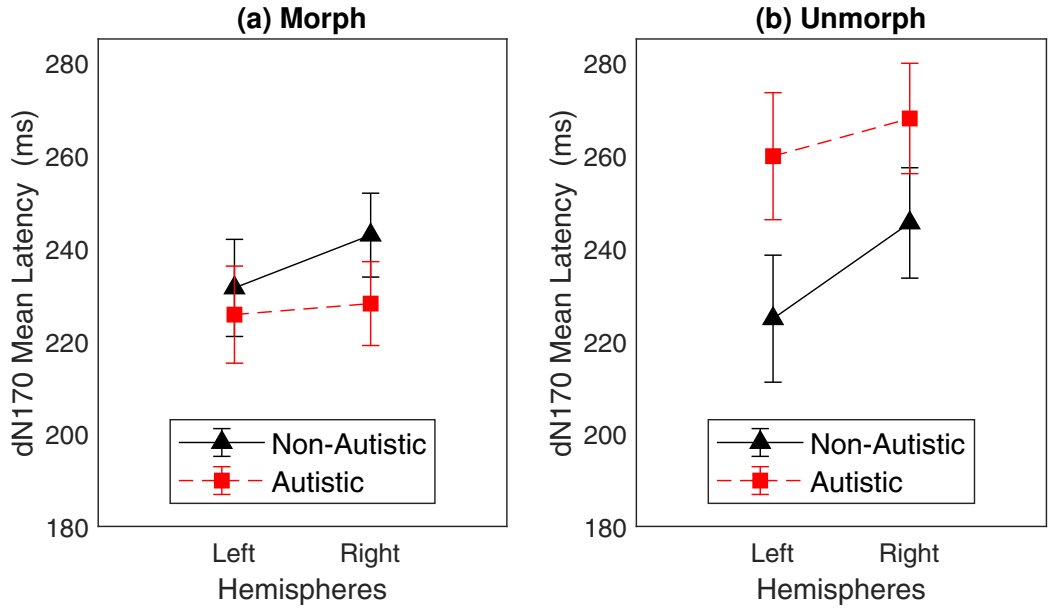

Fig. 3. dN170 latency elicited by dynamic conditions for (**a**) morphing and (**b**) unmorphing by group (non-autistic and autistic) with ± Standard Error of Mean (SEM).

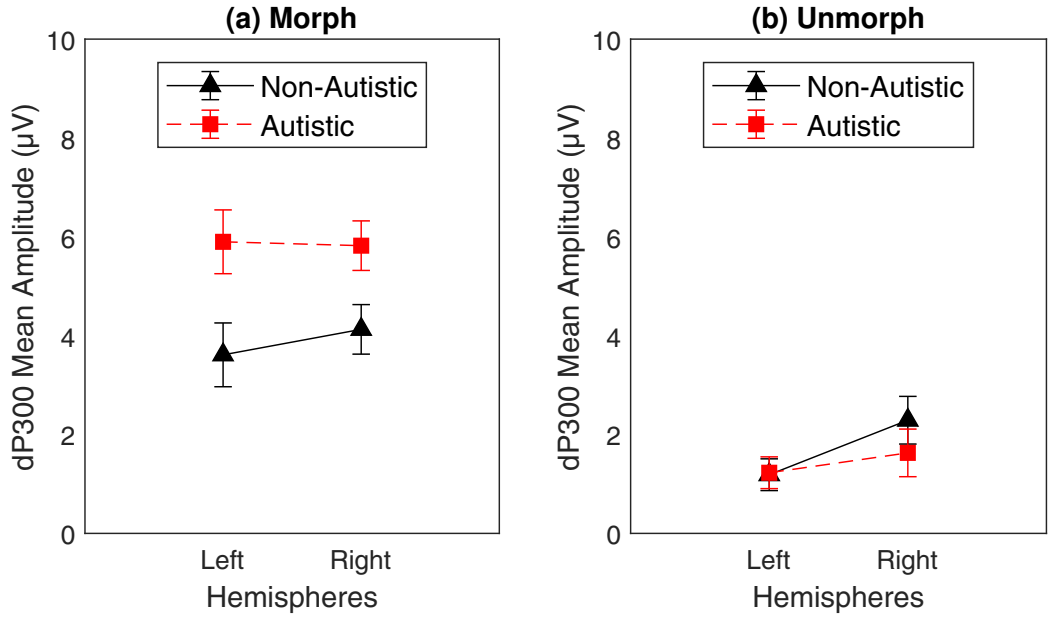

Fig. 4. dP300 amplitude evoked by dynamic conditions for (**a**) morphing and (**b**) unmorphing by group (non-autistic and autistic) with ± SEM.

SE = 8.89) under unmorphed conditions (*95% CI* 3.07 to 54.43, $p = .029$), with no significant difference under the morphed condition. Furthermore, a main effect of dynamic condition was found, $F(1, 30) = 4.34$, $p = .046$, $\eta p^2 = 0.13$, with longer dN170 latencies observed in the unmorphing condition ($M = 249.44$, $SE = 6.29$) compared to the morphing condition ($M = 231.94$, $SE = 5.99$) (Supplementary Material, Figure S2).

Hypothesis 2 (H2): dP300 signature at the amplitude level.

Pertaining to dP300 amplitude, a statistically significant two-way interaction between dynamic condition and group was found, $F(1, 30) = 11.57$, $p = .002$, $\eta p^2 = 0.28$ (Fig. 4 and S4). Post-hoc comparisons revealed higher dP300 amplitudes in the autistic group ($M = 5.86$, $SE = 0.47$) compared to the non-autistic group ($M = 3.87$, $SE = 0.47$) during the morphing condition (*95% CI* 0.64 to 3.34, $p = .005$). This suggests that the autistic group

may be deploying more attentional resources during dynamic facial emotion recognition as a compensatory mechanism. Additionally, a main effect of dynamic trajectory conditions was found, $F(1, 30) = 94.19$, $p < .001$, $\eta p^2 = 0.76$, indicating a higher dP300 amplitude in the morphing condition ($M = 4.87$, $SE = 0.33$) compared to the unmorphed condition ($M = 1.58$, $SE = 0.21$).

In relation to dP300 latency and dynamic conditions, no statistically significant interactions with the group were found. However, a main effect of hemisphere was identified, $F(1, 30) = 9.59$, $p = .004$, $\eta p^2 = 0.24$, with shorter dP300 latencies observed in the left hemisphere ($M = 491.53$, $SE = 14.12$) compared to the right hemisphere ($M = 542.66$, $SE = 12.24$).

Correlation analysis

We investigated correlations between neurophysiological signatures and social communication profiles within the entire study group (i.e., both autistic and non-autistic) to test the hypothesis of a spectrum (H3). Therefore, we found that dN170 amplitudes evoked by the morphing ($rho = -0.42$, $p = .017$, $N = 32$, Benjamini Hochberg $p\text{-}value = 0.224$) and unmorphing ($rho = -0.57$ $p < .001$, $N = 32$, Benjamini Hochberg $p\text{-}value = 0.016$) conditions in the right hemisphere were negatively correlated with the SCQ - reciprocal social interaction domain scores (SCQ-RSI). Furthermore, dN170 amplitudes elicited by the unmorphing in the right hemisphere were negatively associated with the SCQ - total score (SCQ-T), $rho = -0.41$, $p = .021$, $N = 32$ (Benjamini Hochberg $p\text{-}value = 0.224$). In sum, these findings suggest that individuals who had higher dN170 amplitudes evoked by the dynamic conditions in the right hemisphere exhibited fewer social interaction difficulties and less overall social communication differences. These results thus support a right hemispheric lateralisation related to a specialised facial emotional processing system.

Concerning the dP300, we observed that the amplitudes elicited by the morphing condition in the left hemisphere were positively correlated with SCQ - communication domain (SCQ-C), $rho = 0.46$, $p = .008$, $N = 32$ (Benjamini Hochberg $p\text{-}value = 0.192$) and the SCQ-T, $rho = 0.41$, $p = .019$, $N = 32$ (Benjamini Hochberg $p\text{-}value = 0.202$). These relationships indicate that higher dP300 amplitudes in the left hemisphere are associated with poorer social communication skills (or the presence of elevated autistic traits). These results provide support for the attentional compensatory mechanism hypothesis. Also, dP300 morphing amplitudes in the right hemisphere were positively associated with the presence of repetitive, restrictive and stereotyped behaviours (SCQ-R/SB), which did not survive FDR correction ($rho = 0.37$, $p = .037$, $N = 32$; Benjamini Hochberg $p\text{-}value = 0.296$). Nevertheless, a negative correlation was found between dP300 amplitude evoked by unmorphing in the left hemisphere and the SCQ-R/SB, $rho = -0.44$, $p = .012$, $N = 32$ (Benjamini Hochberg $p\text{-}value = 0.192$). These two distinct correlations suggest that, on one hand, higher dP300 amplitudes in the right hemisphere during morphing were associated with more repetitive, restrictive, and stereotyped behaviours. On the other hand, the opposite pattern was observed in the contralateral hemisphere and dynamic condition (i.e., elevated dP300 amplitudes evoked by the unmorphing in the left hemisphere being linked to fewer reported repetitive, restricted, and stereotyped behaviours).

Finally, by assessing the correlations between neural responses and neurometabolite profiles, we found that dN170 latencies elicited by morphing in the left hemisphere were negatively correlated with Glx, $r = -.40$, $p = .049$, $N = 25$. However, this result did not survive FDR correction. In addition, a positive association between GABA concentration in occipital areas and dP300 amplitudes in the right hemisphere during morphing was found, $r = .53$, $p = .007$, $N = 25$. Notwithstanding, a negative association was found between dP300 latencies in the right hemisphere during morphing and Glx concentrations, $r = -.53$, $p = .006$, $N = 25$. These findings suggest that, for the right hemisphere and occipital areas, higher dP300 amplitudes during morphing were linked to augmented GABA levels, while longer latencies were associated with lower Glx concentrations in the occipital area, reflecting an E/I imbalance. Concerning the correlation between neurometabolites and social communication profiles, no statistically significant associations were found.

Discussion

Here we found two novel specific signatures of facial emotional processing in autism . Perceiving and interpreting facial emotional expressions is pivotal in the ability to efficiently process socioemotional contextual information to foster adaptive interpersonal relationships and navigate in daily life[9]. Here, we implemented a dynamic versus static facial expression contrast to isolate emotion processing mechanisms. The paradigm proved useful by creating a contrast against the neutral baseline, by eliminating the P100 component, which only appears in response to the neutral face expressions[28]. Hence, this study investigates the neural responses elicited by an active dynamic facial emotion processing task in autistic and non-autistic children and adolescents. We expected specific group differences related to the third visual pathway, which is proposed to be specialised in the dynamic aspects of social perception[36]and which is activated by our paradigm, due to the specificity of the facial emotion expression contrast[28]. Consequently, this study focused on dynamic temporal trajectories, specifically the transition from a neutral baseline to full expression (morphing) and the reverse transition from full expression back to neutral (unmorphing). Additionally, this study aimed to test hypotheses derived from our previous study[28]which suggested a link between E/I balance, neurophysiological signatures elicited by dynamic facial emotional expressions, and social communication abilities[28].

Our H1 hypothesis was based on previous findings that establish the classical N170 as a potential biomarker and a possible stratification marker for clinical trials with autistic individuals[3]due to its longer latencies during face processing (e.g[15]), indicating slower processing[4]. Concerning dynamic facial expressions, we did find longer dN170 latencies in the autistic group, specifically during the unmorphing condition (i.e., fading of the full expression to the neutral one). Consequently, in the autistic group, the processing of dynamic conditions depended on the temporal trajectory of the facial expression (morph versus unmorph), suggesting a hysteresis effect. Indeed, hysteresis is defined as the dependence of a system's state on its history[41,42]. This effect has been

observed in previous facial emotion recognition studies[41,42]. Therefore, perceptual hysteresis refers to the phenomenon where changes in facial expressions are perceived differently depending on the temporal trajectory, with perception potentially relying on higher-order brain regions[41,42]. We propose that the dependence on temporal trajectory might be due to differential attentional modulation during morphing and unmorphing trajectories in the case of autism. This distinctive neurophysiological signature deserves further investigation. Besides, classical N170 latencies in the right hemisphere elicited by the upright faces were included in the FDA Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program[24,43].

Nevertheless, delays in the classical N170 latency are not specific to autism and have been linked to other neuropsychiatric disorders (e.g., schizophrenia - alterations in the classical N170 amplitude related to face recognition ability)[3]. The dN170 elicited by our paradigm may overcome some limitations of the classical N170, which results from the presentation of static faces[16]. The use of a dynamic and naturalistic paradigm to facial emotion recognition enhances ecological validity by capturing more accurately the neurophysiological signatures relevant for social communication. Furthermore, since our paradigm is based on a specific contrast (presence versus absence of an emotional expression in a face) it evokes a pure face-sensitive dN170, yielding an emotion recognition specific signal. Therefore, future studies using this paradigm should investigate the neural responses it elicits as a putative biomarker and outcome measure for clinical trials. Evidence suggests that shorter classical N170 latencies reflect increased processing speed of holistic faces, stronger adaptive socialisation, and fewer social difficulties[3]. This highlights the notion that the classical N170 may represent a dedicated face-processing system, innately programmed and exclusively engaged in processing faces[3]. Our results corroborate the alterations in this dedicated face-processing system in autistic individuals by providing solid evidence of a delayed neural response. Besides, the hypothesis of a dedicated face-processing system emphasises the dichotomy between nature (i.e., innately programmed) versus nurture (i.e., the role of experience-dependent trained expertise)[3]. Mason and colleagues found evidence of early-stage alterations in face-processing systems that could compromise subsequent development of social cognition[3]. Nevertheless, studies suggest that the classical N170 may be strongly influenced by experience, including expertise–based learning and social interactions[3]. Similarly, facial emotion recognition abilities improve from childhood to adolescence, highlighting how early social experiences can modulate this ability[25]along with later developmental processes[20]. These considerations are relevant, as our sample consists of children and adolescents. Moreover, these insights could inform future interventions, given that training and experience may modulate the neurophysiological mechanisms underlying social communication abilities (e.g[3]).

We could also confirm the H2 hypothesis, which postulated a dP300 with higher amplitudes in the autistic group as a function of morphing of the facial expressions. This finding aligns with the study of Simões et al., which used a similar task of visual and mental imagery[6]. It is known that P300 is an index of top-down processing related to affective and cognitive processes, specifically with elaborative processing of emotional stimuli[9,17]. Also, P300 amplitude appears to reflect the amount of resources required for cognitive processing[21]. Previous studies have shown that naturalistic colour photographs of facial expressions evoked heightened P300 amplitudes, which supported the hypothesis of a compensatory mechanism for facial emotional processing in adults with high autistic traits compared to non-autistic individuals[17]. Therefore, the evidence supports the idea of compensatory strategies in autism, responding to a necessity for a more effortful cognitive processing of facial emotions, contributing to an augmented P300 amplitude[14,17] and longer latencies[9,20,25]. These findings align with neurophysiological differences in social information processing observed in autistic individuals, even in the absence of behavioural impairments in social communication[14,16]. Nevertheless, the inconsistent P300 results across autism studies[6,17,21] raise the question of whether these effects may be context- and time- dependent. It is well-established that the P300 reflects the brain's process of updating its context when exposed to highly salient stimuli[17]. Thus, our findings, showing higher dP300 amplitudes, may reflect a compensatory mechanism that is influenced by context and temporal trajectory.

Autism spectrum has underlying neural processes related to alterations in brain connectivity, structural anomalies, and disruptions in neurotransmitter systems[15]. Based on our previous findings in the non-autistic group[28,] we hypothesised that longer dN170 latencies are associated with lower social communication abilities and that GABA levels are negatively associated with measures of autism characteristics (hypothesis 3). This hypothesis assumes the existence of a continuum of social communication and interaction abilities in non-autistic children and adolescents, who are in a crucial neurodevelopmental period for modulation of social competencies. Therefore, we tested the hypothesis of a dimensional continuum by combining the two groups, and we found that children and adolescents with higher dN170 amplitudes in the right hemisphere had less overall social communication difficulties, which corroborates a right lateralisation associated with a specialised facial emotional processing system[28,37]. These results are therefore in line with our previous work highlighting that worse social communication abilities (therefore more autistic traits within the non-autistic population) were related to longer dN170 latencies[28]. This seems to indicate less efficient or a delayed maturation of the facial emotional processing system[2,9,23]. Additionally, correlation analysis indicated that individuals with higher dP300 amplitudes elicited by morphing in the right hemisphere had elevated GABA concentrations, while individuals with longer latencies had lower Glx concentrations in the occipital brain area, supporting the hypothesis of an E/I imbalance[44]. This aligns with the understanding that brain maturation during the neurodevelopmental period targets sensory pathways, where GABA plays a crucial role in higher-order processes[44]. Moreover, studies have shown how the imbalance of GABAergic processes modify visual processing in autistic and non-autistic adults (e.g[44]). Our results indicate that individuals with elevated autistic traits, such as the presence of repetitive, restricted and stereotyped behaviours, were effectively involving more attentional resources during face processing with the right bias, which supports the compensatory mechanisms[6,20] hypothesis (H2). Previous studies have questioned the hemispheric lateralisation[10,12] while others supported it[6,28,39]. Specifically, a right

bias was reported in autistic individuals with a similar task[6]with complex and realistic social scenes[39]and with our paradigm in individuals with higher levels of autistic traits[28].

Overall, previous evidence has focused on sensory-perceptual alterations in autistic individuals compared to non-autistic individuals[2,10]. Although, we were able to confirm our two previous hypotheses H1 and H2, which led to the question: "Do attentional processing differences dominate over perceptual differences in autism spectrum disorder?" Our findings suggested a neurophysiological difference marked by an attentional overload to decipher the subtle changes in dynamic facial emotion recognition, independently of the specific facial expression. However, this attentional modulation appears to depend on the temporal trajectory of the dynamic expression, which is linked to the third visual pathway implicated in processing dynamic aspects of social perception (e.g., facial movement)[36]. Nevertheless, one cannot overlook the complex neurobiological mechanisms underlying low-level and high-level cognitive processes variations within a continuum from non-autistic to autistic individuals reflecting neurodiversity.

The potential clinical implications of biomarkers derived from the use of a dynamic facial emotional recognition task resembling day-to-day facial emotional expressions should not be underestimated. The use of information stemming from various physiological measures in conjunction with neurochemical profiles and behavioural metrics allows for the profiling of subgroups and more personalised interventions in autistic and non-autistic groups. Hence, from a neurodiversity perspective, it is crucial to combine multiple approaches[45,46]. The childhood and adolescence periods are characterised by an increased efficiency of face emotion processing making this developmental period a target for further studies and interventions based on emotion decoding. One might use the here described dN170 latencies[14] as potential biomarkers in clinical trials with large samples of autistic and non-autistic children and adolescents. The dP300 seems to index compensatory mechanisms associated with the effort of "reading" subtle changes during social communication interactions.

This study's limitations include relatively small sample sizes. Moreover, Harms et al. discussed the importance of matching groups by intellectual ability, since mixed results have been found in facial emotion recognition studies[20]. Gender and sex differences in autism should be addressed in the future, since studies have reported sex-specific patterns in face processing (e.g., autistic females showed increased social attention than males depending on the context[47]; sex pattern differences in autistic females during face processing[48]). Therefore, future studies should consider these aspects and incorporate longitudinal designs, taking into account developmental and age-related alterations in face emotion recognition processing in autistic and non-autistic individuals[20,25]. New methodologies and advances can be useful to deepen the understanding of our findings, such as the use of a Ultra-high field (7 Tesla) MRI, which has better signal to noise ratio to measure GABA, for example, using sequences such as MEGAPRESS (e.g[27]) or HERCULES, which is an approach allowing simultaneous editing and multiplexed fitting of up to seven low-concentration and six high-concentration metabolites in a single 11-minute 3 T acquisition enabling a broader clinical application of edited MRS (e.g[49]). This integration of distinct and complementary methodologies is crucial to better understand social communication mechanisms, since there are autistic individuals who exhibit reasonable emotion recognition abilities[14]. Additionally, future studies should include neuropsychological assessments of executive functions and attention, as these may affect ERPs responses. Also, future approaches can use variations of this paradigm with a broader set of emotions (e.g., complex and ambiguous) with larger samples, and addressing other neurodevelopmental disorders (e.g., intellectual disability or attention-deficit/hyperactivity disorder) to study the extension and specificity of neurophysiological differences identified in autism.

In sum, the paradigm we employed increased ecological validity and allowed for a more naturalistic approach to socioemotional cognition, helping to elucidate the roles of specific face expression-sensitive dN170 and dP300 in autism and across the spectrum from non-autistic to autistic individuals. Our findings provided evidence of this dynamic facial expression recognition task in differentiating individuals across the autism spectrum continuum (from non-autistic to autistic), suggesting it could serve as a potential biomarker.

Methods

Power analysis

A power analysis was conducted based on the results previously reported by Simões et al.[6]. We obtained a total sample size of 22 participants (which means 11 participants per group) to detect significant differences with an alpha level of 0.05 and 80% statistical power (G*Power3.1).

Study population

The study comprised two groups of participants. All participants gave assent, and written informed consent was obtained from their parents and/or legal guardians. The study was approved by the ethics committee from the Faculty of Medicine (CE-10/2017), the University of Coimbra (UC, Portugal), and the Centro Hospitalar e Universitário de Coimbra (CHUC-024-18) (Portugal). The study was conducted following the Declaration of Helsinki. Sixteen autistic children/adolescents (11 males and 5 females; mean (*M*) age = 13.31 ± 2.18 years) were recruited from the Neurodevelopmental and Autism Unit, Child Developmental Centre, Paediatric Hospital, Unidade Local de Saúde de Coimbra (ULS de Coimbra, Portugal). Autism diagnosis was assigned based on the gold standard instruments: parental or caregiver interview, Autism Diagnostic Interview–Revised (ADI-R)[33] and direct semi-structured proband assessment with the Autism Diagnostic Observation Schedule (ADOS)[50]; and clinical examination performed by an experienced neurodevelopmental paediatrician, based on the current diagnostic criteria for autism spectrum disorder according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition, DSM-5[1]. All autistic children/adolescents had positive results on at least one of the administered instruments for autism or autism spectrum disorder. They met the clinical criteria for autism spectrum as defined by the DSM-5. The other group comprised 16 non-autistic children/adolescents (11 males and 5 females; *M* = 13.31 ± 1.92 years) recruited from our local volunteers' database, schools, and the community

in Coimbra (Portugal). A clinical psychologist completed a clinical interview with all caregivers of autistic and non-autistic children/adolescents and administered the Social Communication Questionnaire[40] (SCQ, see Supplementary Material) as a screening tool for autism spectrum (exclusion criteria for inclusion in the non-autistic group) and to evaluate their children's social communication abilities. Participants in the non-autistic group had no reported history of either neurodevelopmental or neurological disorders. All children/adolescents underwent a neuropsychological assessment with the Wechsler Intelligence Scale for Children – 3rd Edition (WISC-III)[51] or Wechsler Intelligence Scale for Adults – 3rd Edition (WAIS-III)[52] allowing the determination of the full-scale intellectual quotient (FSIQ), verbal IQ (VIQ) and performance IQ (PIQ) and completed the Edinburgh Handedness Inventory (EHI)[53]. Table S1 (Study Population, Supplementary Material) shows the groups' characterisation based on sociodemographic data and neuropsychological assessment.

Facial emotion recognition task

The visual Facial Emotion Recognition Task was previously reported in Sousa et al.[28]. This task consists of a set of trials, each beginning with a baseline period (black screen with fixation dot) for 1–1.5 s, then a virtual avatar displayed a neutral expression (1 s), followed by a morphing period of 250 ms where the avatar gradually transitions to the target facial expression (happy or sad) displaying in its full extent (1 s), and a final period of 250 ms where the avatar morphs back (unmorphed) to the neutral expression (1 s) (Fig. 1b). This task includes three parameters to ensure unpredictability, namely: (1) randomisation of emotional expressions, (2) distinct randomisation within each run, and (3) each condition (sad or happy) could only appear in a maximum of three consecutive trials. The participants were prompted to fixate on the face of the avatar in the middle of the eyes, observe the expressions, and decide whether the avatar displayed a happy or sad expression by pressing one of two buttons (Fig. 1a). This experiment consisted of three runs of roughly 4 min (50 trials per run – 25 for each expression: sad and happy), with short breaks between runs to ensure focus and reduce fatigue during the task. The experiment lasted approximately 40 min, from preparation to completion.

Electroencephalography (EEG) acquisition and processing

This experiment was carried out on a 22-inch LCD monitor (frame rate of 60 Hz, 1680 × 1050 pixel resolution) and the paradigm was implemented using Matlab® (*Mathworks*, *version R2017a*). The participants were approximately 60 cm away from the screen.

EEG signals were acquired with an actiCAP cap of 64 Ag/AgCl active electrodes, according to the international 10–10 standard system directly linked to the Brain Products actiCHamp amplifier and sampled at 1000 Hz (electrode impedance was kept under 15 kΩ). The ground electrode was located at AFz and the reference electrode was at FCz position. EEG data were recorded using the BrainVision Recorder software (*Brain Products*, *version 1.20.0801*).

Data pre-processing and analysis were performed using Matlab® (*Mathworks*, *version R2019b*) and the EEGLAB toolbox v2019_0[54]. EEG signals were filtered with a finite response bandpass filter with lower and higher cut-off frequencies set to 0.1 and 30 Hz, respectively. Then, bad channels were removed by visual inspection (in the non-autistic group 3.62% of the channels were removed, and in the autistic group 6.91% - below or slightly above the standard 5% limit[55]) and then interpolated. Subsequently, data were re-referenced to the common average reference. Epochs were defined as time-locked to the onset of the facial expression morphing and unmorphing, beginning 2 s before and lasting up to 3.5 s after. Bad epochs were eliminated based on the EEGLAB semi-automatic procedures for extreme values and improbable signal segments. On average 95.79% and 81.04% of the trials remained for further analysis in the non-autistic and autistic groups, respectively. Independent Component Analysis (ICA) was subsequently performed on data using EEGLAB's implementation of the infomax algorithm[56] to extract noisy components (e.g., blinks, muscular activity). These components were labelled using the EEGLAB plug-in ICLabel, removed, and the weights of the remaining components were projected back to the data[57]. Then, an analysis of EEG data was carried out.

ERPs were computed across lateral posterior-temporal sites (P3, P4) with ERPs N170 and P300 windows defined based on our previous study[28] (i.e., windows of 150–300 ms and 300–700 ms were defined for dN170 and dP300, respectively) by averaging trials grouped by dynamic conditions (baseline correction of 200 ms before the stimulus onset). The peak values were automatically identified as local minima for negative waves, or local maxima for positive waves. Then, grand average peak amplitudes and latencies for each component were extracted for each participant. In the previous study[28] the dN170 peaked on average at 249 ms and 252 ms for morphing and unmorphing, respectively while the dP300 peaked with a delayed latency (on average) at 535 ms and 557 ms for the morphing and unmorphing conditions, respectively.

Acquisition of magnetic resonance imaging (MRI) data

MRI acquisitions were carried out using a 3 T Siemens Magnetom Prisma MRI Scanner (Siemens, Erlangen, Germany) at the Institute for Nuclear Sciences Applied to Health (ICNAS) of the University of Coimbra (Portugal). A high-resolution T1-weighted three-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence [repetition time (TR) 2530 ms, echo time (TE) 3.5 ms, inversion time (TI) 1100 ms, flip angle (FA) 7º, the field of view (FOV) 256 × 256 mm^2 yielding 192 slices with 1 × 1 × 1 mm^2 voxel size and 1 mm of thickness] was firstly performed for structural assessment and localised 1 H-MRS voxel placement for each participant. 1 H-MRS data were acquired on a volume located in the occipital cortex (voxel size: 30 mm x 30 mm x 30 mm), positioned according to sagittal, coronal, and axial planes to minimize partial volume effects. GABA and Glx (Glutamate + Glutamine) measurements were performed with the Hadamard Encoding and Reconstruction of Mega-Edited Spectroscopy (HERMES) approach[58] as implemented with the following parameters: TR = 2000 ms, TE = 80ms, number of averages = 320, flip angle = 90º, bandwidth = 2000 Hz. An unsuppressed water signal (TR = 2000 ms, TE = 80 ms, number of averages = 32, flip angle = 90º, bandwidth = 2000 Hz) was collected

immediately after acquiring the water-suppressed spectrum. The participants watched videos during the scanning protocol to help them remain still for the approximately 20-minute acquisition time.

1 H-MRS data processing and quality check

The Gannet toolbox v3.0[59] with a default pipeline using Matlab® (*MathWorks*, *version R2019a*) was employed to process TWIX file data. Spectra were first inspected for movement artefacts and corrected for frequency drift. A difference spectrum was created per participant and peak integration was used to quantify GABA (3.0 ppm) and Glx (3.75 ppm). Here, the GABA signal is identified as GABA + to indicate the potential contribution of macromolecules and homocarnosine at 3.02 ppm[60]. Integrals of GABA+, Glx, and total creatine (tCr) peaks were automatically calculated using a Gaussian (GABAþ), Gaussian doublet (Glx) and Lorentzian (tCr) models to best fit the peaks, as executed by the toolbox. Relative proportions of grey matter, white matter, and cerebrospinal fluid within the voxel, were achieved by performing tissue segmentation of T1-weighted images using the same software and SPM12 toolbox (http://www.fil.ion.ucl.ac.uk/spm). This segmentation information was then used to adjust metabolite levels to correct for different voxel compositions. Also, segmentation reduces inter-subject variability attributable to differences in signal-to-noise ratio, regional susceptibility variations, and cerebrospinal fluid fraction within the voxel[61]. Ultimately, absolute quantification of GABA and Glx concentrations were taken relative to water peak, therefore expressed in institutional units (i.u.). The final sample size in the non-autistic group was 11 individuals, and in the autistic group, it was 14 children and adolescents, due to poor signal-to-noise ratio.

Statistical analysis

All statistical analyses were performed in IBM Statistical Package for the Social Sciences (SPSS), Version 28. The normality assumption was verified using the Shapiro-Wilk test. Most ERP parameters were normally distributed. Hence, a three-way mixed Analyses of Variance (ANOVA) was performed. Greenhouse-Geisser corrections were used for violations of the sphericity assumption. The Bonferroni adjustment for multiple comparisons was applied to maintain the level of statistical significance. We investigated the ERPs (amplitude and latency) elicited by a morphing avatar of facial expressions between two groups: autistic versus non-autistic children and adolescents (between-subjects factor) and with dynamic conditions (morph and unmorph) and hemispheres (P3 or P4 sites) as within-subjects factors. Finally, to investigate the ERPs and neurochemicals relationships (the ratio between GABA/Glx was calculated) with the participant's social communication skills measured by SCQ, Spearman's rho correlations and/or Pearson correlations were performed. The False Discovery Rate (FDR) using the Benjamini-Hochberg procedure was also implemented for multiple comparisons correction, with a critical value of 0.25[62]. An alpha level of 5% was used as the statistical significance threshold for all the statistical tests conducted.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Author contributions
DS: Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Visualization. AF: Investigation, Writing – review & editing. HP: Investigation, Validation, Visualization, Writing – review & editing. JA: Investigation, Validation, Writing – review & editing. JC: Investigation, Validation, Writing – review & editing. MS: Investigation, Validation, Writing – review & editing, Methodology, Software. RM – Software, Writing – review & editing; SM – Investigation, Writing – review & editing; FD - Investigation, Writing – review & editing; GO - Investigation, Writing – review & editing; MCB: Investigation, Validation, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – original draft.

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Declarations

Competing Interests
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