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# Gaze behavior, facial emotion processing, and neural underpinnings: A comparison of adolescents with autism spectrum disorder and conduct disorder

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**Background:** Facial emotion processing deficits and atypical eye gaze are often described in individuals with autism spectrum disorder (ASD) and those with conduct disorder (CD) and high callous unemotional (CU) traits. Yet, the underlying neural mechanisms of these deficits are still unclear. The aim of this study was to investigate if eye gaze can partially account for the differences in brain activation in youth with ASD, with CD, and typically developing youth (TD). **Methods:** In total, 105 adolescent participants ($N_{CD}$ = 39, $N_{ASD}$ = 27, $N_{TD}$ = 39; mean age = 15.59 years) underwent a brain functional imaging session including eye tracking during an implicit emotion processing task while parents/caregivers completed questionnaires. Group differences in gaze behavior (number of fixations to the eye and mouth regions) for different facial expressions (neutral, fearful, angry) presented in the task were investigated using Bayesian analyses. Full-factorial models were used to investigate group differences in brain activation with and without including gaze behavior parameters and focusing on brain regions underlying facial emotion processing (insula, amygdala, and medial prefrontal cortex). **Results:** Youth with ASD showed increased fixations on the mouth compared to TD and CD groups. CD participants with high CU traits tended to show fewer fixations to the eye region compared to TD for all emotions. Brain imaging results show higher right anterior insula activation in the ASD compared with the CD group when angry faces were presented. The inclusion of gaze behavior parameters in the model reduced the size of that cluster. **Conclusions:** Differences in insula activation may be partially explained by gaze behavior. This implies an important role of gaze behavior in facial emotion processing, which should be considered for future brain imaging studies. In addition, our results suggest that targeting gaze behavior in interventions might be potentially beneficial for disorders showing impairments associated with the processing of emotional faces. The relation between eye gaze, CU traits, and neural function in different diagnoses needs further clarification in larger samples. **Keywords:** Conduct disorder; autism spectrum disorders; emotion processing; eye gaze; brain activation.

## Introduction

Facial emotion processing is a fundamental skill for prosocial behavior. Already from very early in life, babies display a natural preference to look at faces (Goren, Sarty, & Wu, 1975; Valenza et al., 2019). The eye region is described as a main source of social information, nonverbal social interaction, and learning (Adams & Nelson, 2016; Batki, Baron-Cohen, Wheelwright, Connellan, & Ahluwalia, 2000; Hamilton, 2016; Mundy & Newell, 2007). Thus, attention to the eyes is crucial for the accurate processing of facial expressions (Schindler & Bublatzky, 2020).

Autism spectrum disorders (ASD) are defined by restricted interests, deficits in social interaction and social communication, and reduced empathic responsiveness (American Psychiatric Association, 2013). Children and youth with conduct disorder (CD) are characterized by severe and persistent antisocial and/or aggressive behaviors and difficulties in following rules or acting in a socially acceptable way (American Psychiatric Association, 2013; Frick & Nigg, 2012). Those with elevated callous unemotional traits (CU traits) defined by a lack of remorse, empathy, and shallow affect, are at specific risk for chronic maladjustment (Frick & White, 2008).

Although ASD is a heterogeneous disorder linked to varying genetic and neurobiological factors (Constantino, Charman, & Jones, 2021; Johnson

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et al., 2021; Klin et al., 2020), reduced eye contact is one of the early signs for ASD (Constantino et al., 2021; Klin et al., 2020), linked to deficits in emotion processing and social functioning (Riddiford, Enticott, Lavale, & Gurvich, 2022). Reduced fixation to the eyes has been especially reported in children and adolescents with CD or with conduct problems and high CU traits, particularly when negative emotions are presented (Díaz-Vázquez, López-Romero, & Romero, 2024). Furthermore, the association between CU traits and reduced eye gaze might even be observed from early childhood (Bedford et al., 2017; Bedford, Pickles, Sharp, Wright, & Hill, 2015). In addition, reduced eye gaze has been hypothesized to potentially underlie the facial processing deficits described in young children with high CU traits (Dadds et al., 2014; Dadds, El Masry, Wimalaweera, & Guastella, 2008).

Although it is well established that altered facial emotion processing involves interactive brain networks (Vuilleumier & Pourtois, 2007), their disorder-specific neural underpinnings are still unclear. In ASD, different theories suggest that reduced eye gaze is based on opposite neural functioning responses in the amygdala. On the one hand, the amygdala theory of autism suggests lack of eye contact is caused by a hypoactivity in the amygdala (Baron-Cohen et al., 2000). On the other hand, the eye avoidance hypothesis proposes that the altered eye gaze behavior often described in ASD participants is based on amygdala hyperactivity (Tanaka & Sung, 2016). Accordingly, it has been shown that ASD participants exhibit higher unpleasant arousal in response to eye contact than neurotypicals, coupled with amygdala hyperactivity (Tanaka & Sung, 2016). As a strategy to reduce this unpleasant hyperarousal, direct eye contact is avoided, which would be consistent with measures of individuals on the autism spectrum stating that direct eye gaze induces stress and anxiety (Trevisan, Roberts, Lin, & Birmingham, 2017), and a recent meta-analysis suggesting that atypical amygdala activation is linked to eye avoidance (Stuart, Whitehouse, Palermo, Bothe, & Badcock, 2023). Taken together, in youths with ASD, atypical eye gaze behavior might be linked to altered brain functioning especially in the amygdala.

Atypical activity in the amygdala, as well as in the insula and orbitofrontal cortex (OFC) during facial emotion processing has been reported in youth with CD and high CU traits (Berluti, Ploe, & Marsh, 2023; Jones, Laurens, Herba, Barker, & Viding, 2009; Viding et al., 2012). CU traits have been linked to hypoactivity in the amygdala in response to fearful facial expressions (Lozier, Cardinale, VanMeter, & Marsh, 2014) supporting theories that those with high CU traits display reduced empathic responses to emotional distress cues (Jones et al., 2009; Marsh & Blair, 2008; Viding et al., 2012). This also highlights the relevance of CU traits as an important phenotype for empathic responses and atypical brain activation patterns in CD. Thus, amygdala dysfunction has been linked to atypical gaze behavior and facial emotion processing deficits both in ASD and CD, indicating potentially interrelated underlying neural mechanisms in these disorders.

Some initial evidence showed that, compared to healthy peers, fixations to the eyes are reduced both in youth with ASD and in youth with CD, suggesting a potential overlap in differential gaze behavior (Bours et al., 2018). However, the evidence on the compared neural correlates of emotion processing in both disorders is scarce. Klapwijk et al. (2016) investigated brain function in ASD and CD with elevated CU traits during emotion processing, and results suggest disorder-specific abnormal brain function associated with specific aspects of empathic functioning. During an emotion recognition task, and relative to healthy peers, only youth with ASD showed reduced ventromedial prefrontal cortex (vmPFC) responses. On the other hand, during emotional resonance processing, the ASD group showed reduced responses in the hippocampus, while the CD group showed lower brain responses in the anterior insula and the left inferior gyrus. However, both groups shared reduced amygdala response relative to healthy comparison participants (Klapwijk et al., 2016). Interestingly, Menks et al. (2021) assessed concurrent eye gaze behavior during emotion processing in CD participants and typically developing youth, and results revealed that neural group differences in the insula are reduced when controlling for eye gaze behavior.

In sum, there are various studies indicating that behavioral (gaze behavior) and neural (brain activation) differences during facial emotion processing are present in both youth with CD and youth with ASD. However, studies comparing the shared and disorder-specific character of the association between gaze behavior and neural activation during facial emotion processing in ASD and CD populations are lacking. Thus, the objective of the current study is to investigate emotion processing in both disorders and, by assessing concurrent eye-tracking, we aim to answer the question of whether neural function in facial emotion processing is linked to differences in eye gaze behavior.

At the behavioral level, we hypothesize a reduced number of fixations to the eyes in youth in the ASD and CD groups independently of the emotion presented than the TD group. Furthermore, we expect the levels of CU traits to be inversely correlated with the number of fixations to the eye region across both disorders. At the neural level, we expect that both youth in the ASD and CD groups would show reduced vmPFC activation compared to the TD group (TD > CD, ASD) and that youth in the CD group would show reduced insula and amygdala activation compared to the TD group and youth in the ASD group during facial emotion processing (CD < TD, ASD). Finally, we hypothesize that the relationship between facial emotion processing and neural activation in the insula, amygdala, and vmPFC is influenced by the number of fixations to the eye/mouth region. We, thus, expect neural differences to be partly reduced when controlling for the number of fixations.

## Methods

### Participants

Adolescent participants with ASD and participants with CD were recruited from different specialized clinical settings and residential centres in Basel and Zurich, while TD participants were recruited from socioeconomically diverse secondary schools in Canton Basel-Stadt. Participants in the ASD and CD groups needed to fulfill the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013) for either diagnosis, with no comorbid depressive or anxiety disorder or comorbid ASD and CD diagnoses. The requirement for inclusion in the TD group was no current or previous diagnosis of any psychiatric disorder. A semi-structured clinical interview (Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version, K-SADS-PL; Kaufman et al., 1997) was conducted to evaluate diagnostic inclusion criteria. For the ASD group, the Autism Diagnostic Observation Schedule (ADOS) or Autism Diagnostic Interview – revised (ADI-R; Bölte, Poustka, Rutter, & Bölte, 2006; Poustka et al., 2015) was additionally administered. CD and other common psychiatric disorders were assessed using the Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997). Of all participants with CD, 40 participants met the diagnostic criteria for current CD. Within the CD group, 10 participants additionally fulfilled criteria for oppositional defiant disorder (ODD) while in the ASD group, two participants additionally fulfilled ODD criteria. Furthermore, only participants with an IQ score in the normal range (>70) were included in the study. Due to the IQ criteria, the ASD group consequently consisted of youth who fulfilled diagnostic criteria for either Asperger's syndrome or high functioning autism. Participants underwent a functional brain imaging data acquisition session, and caregivers filled out reports on attention problems (subscale of the Childhood Behavior Check List; Achenbach, Dumenci, & Rescorla, 2001) and CU traits (Inventory of Callous Unemotional traits, ICU; Frick, 2017). Written informed assent and/or consent was obtained from participants and caregivers. All procedures were conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee in Basel, Switzerland (EKNZ: Ethikkommission Nordwest- und Zentralschweiz, 2019-02386).

The initial sample consisted of 125 adolescent participants of which 20 were excluded from analysis due to poor gaze data quality. Thus, the final sample included in the gaze behavior analysis consisted of 105 adolescents ($N_{CD} = 39$, $N_{ASD} = 27$ and $N_{TD} = 39$) aged 10–18 years ($M = 15.59$; $SD = 1.97$ years). For the functional Magnetic Resonance Imaging (fMRI) analysis, data from three additional participants had to be excluded due to incomplete data ($N_{CD} = 2$, $N_{TD} = 1$). Thus, the final imaging sample consisted of 102 participants ($N_{CD} = 37$; $N_{ASD} = 27$; $N_{TD} = 38$). The data were collected in two waves from 2015 to 2023 with a time lag of 18 months between waves. Data of 57 participants were collected in the first ($N_{CD} = 21$; $N_{ASD} = 2$; $N_{TD} = 34$) and 48 ($N_{CD} = 18$; $N_{ASD} = 25$; $N_{TD} = 5$) in the second wave. Additionally, for the presentation of the implicit facial emotion processing task, different programs were used: Presentation® software (Neurobehavioral System, Inc.) in the first, and E-Prime® in the second data collection wave. To account for potential differences during data collection, we included data collection wave as an additional regressor of no interest in all analyses to account for potential differences during data collection. Further details on the sample characteristics are shown in Appendix S1.

### fMRI implicit facial processing task

An adapted version of the implicit emotional face processing paradigm from Passamonti et al. (2010) was used after successful application in previous studies (Fairchild et al., 2014; Menks et al., 2021; Passamonti et al., 2010). The task consisted of two runs (length per run: 8.25 min, total length: 16.5 min). Participants were instructed to indicate the gender of each face and to fixate on the white crosses shown in between stimuli (see Figure S1). For each facial stimulus, gaze behavior (number of fixations and fixation duration) and task performance were recorded (for more information on task characteristics see; Menks et al., 2021). Descriptive analysis results per group for task performance can be found in Appendix S1.

### Eye tracking data acquisition

The eye-tracking device model of VisualSystem USB 60x3 produced by Nordic NeuroLab, Bergen, Norway was used with the ViewPoint software by Arrington Research®. Eye movements were recorded at a rate of 60 Hz. The device was attached to the right ocular of the binocular video goggles. The raw data provided by the interface was in the form of pairs of coordinates identifying the pupil location in the field of view of the camera. Monitor distance was 10 cm with a size of 14.3 by 10.7 cm and a resolution of 800 × 600 pixels. To ensure an equal eye-tracking starting position for each trial, a fixation cross was located on the nose bridge in between the eyes and mouth location of the stimuli during the fixation trials. Prior to recording, the camera was adjusted to ensure clear vision for the participant and was centrally located at the participant's right eye for satisfactory data recording. At the beginning of each run, a 9-point calibration was performed. Calibration was repeated until satisfactory.

### Functional imaging data acquisition

Whole-brain functional magnetic resonance images were obtained using a 3 T MR imaging system (Siemens Prisma) and a 20-channel phased-array radio frequency head coil. After automatic second-order shimming of the magnetic field, functional whole-brain volumes were acquired using a T2*weighted echo-planar imaging (EPI) sequence: TR = 2,500 ms; TE = 30 ms; flip angle = 83°; FoV = 192 mm; 41 slices; matrix size = 64 × 64; and voxel size = 3 × 3 × 2 $mm^3$. To allow a steady magnetization before the task started, the first four functional time points of each run were discarded. Additionally, a high-resolution T1-weighted Magnetization-Prepared Rapid Acquisition Gradient Echo (M-PRAGE) dataset was acquired with the following parameters: TR = 1,900.0 ms; TE = 3.42 ms; flip angle = 9°; FoV = 256 mm; matrix size = 256 × 256; and voxel size = 1 × 1 × 1 $mm^3$.

### Gaze behavior analysis

The raw data were processed in Matlab as well as the EyeMMV toolbox (Krassanakis, Filippakopoulou, & Nakos, 2014) and analyzed using R (Version 4.2.1, RStudio Version 2022.7.0.547; R Core Team, 2020; RStudio Team, 2022). Two areas of interest (AOI) were defined. One AOI concentrated on the eyes of each facial stimulus, and the other AOI concentrated on the mouth. Both AOIs were rectangular and the same size. Positions of the AOIs on the screen were the same across all stimuli. The main measures of gaze behavior are the number of fixations on the eye region and on the mouth region per emotion and across all emotions. Fixations were defined using the fixation identification algorithm implemented in the EyeMMV (Krassanakis et al., 2014) as having a minimum duration of 150 ms and using the parameters $t1 = 0.05$, $t2 = 0.1$, $minDur = 150$, $maxx = 1$, $maxy = 1$. Blinks and offscreen events were removed before calculating fixations, and gaze data during each trial was corrected for head movements. To ensure data quality, participants with poor gaze data were removed from the analysis. Participants were excluded if more than 60% of their trials had <500 ms of usable gaze data, the calibration failed, or if the eye tracking system malfunctioned. The number of fixations was calculated by dividing the fixation count for each AOI by the total fixation count for the entire screen. The dependent variables were calculated for each trial and then averaged for each emotion (neutral, angry, or fearful). Likewise, fixation duration was also calculated by dividing the duration of fixations within each AOI by the total duration of fixations on the entire screen.

From the whole dataset, missing data represent 6.67% of the ICU scores and 26.67% of the CBCL scores. Using the 'mice' package, multiple imputation by chained equations was implemented (van Buuren & Groothuis-Oudshoorn, 2011; m = 100, maxit = 20, meth = 'pmm') to maximize the data used for those analyses. We investigated group differences in gaze behavior (number of fixations to the eyes/mouth) using two models within a Bayesian multilevel framework, with the group values entered as the main regressor of interest and gaze behavior (number of fixations to the eyes/mouth) as the dependent variable for the emotions: angry, fearful, and neutral. The models included age, sex, IQ, the CBCL attention problem subscale scores, and data collection wave as regressors of interest. Additionally, the total sum scores of the ICU were added to investigate the hypothesized interaction effect of the CD group and co-occurring CU traits on gaze behavior. Models included a flat prior and a Gaussian likelihood distribution, with parameters warmup = 2,000, iter = 4,000, four chains, and three cores. All variables were standardized before being entered in the analyses. Additionally, supplementary analysis was conducted for fixation durations on the eye and mouth regions as dependent variables. Correlation analyses were conducted on the potential link between CU traits score and number of fixations to the eye region. To investigate potential differences in slopes in the association between CU traits and fixations to the eye region, the correlational analysis also included an interaction term for the variable group.

### fMRI analysis

Functional neuroimaging data were preprocessed and analyzed in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/), implemented in MATLAB (version 2014b; MathWorks). Preprocessing included data quality check, slice time correction, realignment, co-registration to the structural images, segmentation of the structural image, normalization to MNI space, and smoothing (8 mm FWHM). Based on previous studies (Menks et al., 2021; Passamonti et al., 2010), all analyses were restricted to one mask of binarized and 50%-thresholded regions that was built from six regions of interest (ROI) which included bilateral insula, bilateral amygdala, and vmPFC of the Harvard Oxford Atlas in FSLeyes (Version 1.3.0; see Figure S2). For each participant, blood-oxygen-level-dependent (BOLD) responses were modeled, including six motion regressors to account for motion effects and temporal inhomogeneities, and a global regressor for each run to account for differences between runs. Our first-level model included emotional faces (neutral, anger, fearful) and three fixation conditions (neutral fixation, anger fixation, fearful fixation) as regressors of interest. The following contrasts were created: neutral stimuli > neutral fixation, anger stimuli > anger fixation, and fearful stimuli > fearful fixation (Fairchild et al., 2014). For this full factorial model, group was set as a regressor of interest. Age, IQ, sex, the CBCL attention problems score, and data collection wave were added as regressors of no interest. In this model, the total sum score of the ICU was also added as a covariate. To investigate a possible effect of gaze behavior on brain activation differences, in a second model, the number of fixations to the mouth/eye region per group was included as an additional regressor of interest for each emotion (neutral, anger, fearful). Significant parameters are extracted via FSL, using a mask including the significant cluster region and the respective significant cluster of the randomize fMRI analysis in FSL (Brett, Anton, Valabrgue, & Poline, 2002). All results are reported using a cluster-building threshold of $p < .001$ and a cluster-level correction for multiple comparisons of $p < .05$, familywise error (FWE-) corrected.

## Results

### Sample characteristics

Group comparisons revealed differences in sex, IQ, CBCL attention problems (AP) scores, and ICU sum scores (see Appendix S1). Both the ASD and CD groups showed significantly higher ICU sum scores than the TD group. The results also show significant group differences in task performance accuracy (see Appendix S1). For all emotions, the TD group consistently showed the highest accuracy rates. The number of fixations to the eyes and mouth regions per group is depicted in Figure S3.

### Gaze behavior results

Across all emotions, results showed significant group differences. The ASD group displayed a higher number of fixations to the mouth region than the TD and CD groups (Table 1). A significant negative interaction effect of the CD group and CU traits for eye gaze across all emotions is shown (Table 1). Correlation analysis between the level of CU traits and number of fixations to the eye region further reveals a significant positive correlation in the TD group and a negative correlation trend in participants with CD (Figure 1). Additional analyses testing the significance of correlations between groups revealed significant differences between correlation coefficients of the CD in comparison with the TD group across all emotions and between the ASD and TD group for the emotion fearful (see Appendix S2). The ASD group showed significantly more fixations to the mouth region than both the TD and CD groups. Compared to the TD and CD groups, the ASD group showed significantly more fixations to the mouth region when fearful faces are presented (Table 2). Compared with the TD group, the ASD group also showed significantly more fixations to the mouth region for neutral faces. Compared with the

CD group, the ASD group showed more fixations to the mouth when angry faces are presented (Table 2). Additional analyses show group comparison results on fixation durations to the eyes and mouth regions (see Appendix S3). Findings revealed that the ASD group showed higher fixation durations to the mouth than both the TD group and CD group across all emotions. Although we excluded participants with a diagnosis of depression or anxiety, in a supplementary post-hoc model we accounted for differences in the main results that might be related to subclinical levels of anxiety and depressive symptoms. Results of this model (see Appendix S4) revealed that across all emotions, the ASD group showed more fixations to the mouth compared with the TD and CD groups (see Appendix S4).

### *fMRI results*

Initial *F*-tests showed significant group differences for all emotions in numerous regions, most consistently the bilateral anterior insula (see Appendix S5). For *t*-test group comparisons (TD vs. ASD, TD vs. CD, ASD vs. CD) across all emotions, no results survived multiple comparison corrections. Post-hoc exploratory analysis revealed increased brain activation in the ASD group compared to the CD group in the right anterior insula specifically when processing angry faces (MNI = 33.4; 17.6; 4.9, 385 $mm^3$ volume, $t = 3.91$; Figure 2). When the number of eye and mouth fixations were included in the model, the cluster of activation was smaller (MNI = 32.6; 18.3; 2.7, 157 $mm^3$ volume, $t = 3.84$; Figure 3). Post-hoc Bayesian analysis on the effect of eye gaze on the extracted parameters of the insula cluster showed a positive main group effect for the ASD group when accounting for differences in fixations to the mouth region (see Appendix S6). Activation parameters for the insula cluster per emotion and group are shown in Figure S4. Independent of whether CU traits were included in the model, we observed a significant group difference between the ASD and CD groups (Figure 2; Figure S5). Of note, we found that neural activation differences between diagnostic groups (CD vs. ASD) were increased when CU traits were included in the model, suggesting that their inclusion removes at least part of the variance of brain function that is related to CU traits across both disorders (MacKinnon, 2000). For completeness, all analyses were repeated adopting a whole brain approach with no significant results. To further investigate the potential role of CU traits in group differences in the insula cluster, we conducted supplementary Bayesian analysis including an

**Table 1** Bayes multilevel regression results

| | Estimate | Est. error | l-95% CI | u-95% CI | Rhat | Bulk_ESS | Tail_ESS |
|---|---|---|---|---|---|---|---|
| Fixations to the eyes | | | | | | | |
| **Angry faces** | **−0.23** | **0.06** | **−0.34** | **−0.12** | **1** | **5,521** | **5,730** |
| Fearful faces | −0.07 | 0.06 | −0.18 | 0.04 | 1 | 5,874 | 5,591 |
| ASD | −0.29 | 0.42 | −1.11 | 0.53 | 1 | 1,200 | 1,837 |
| CD | −0.49 | 0.33 | −1.18 | 0.15 | 1 | 1,147 | 1,969 |
| **CU traits** | **0.47** | **0.21** | **0.05** | **0.89** | **1** | **1,029** | **2,234** |
| Male sex | −0.24 | 0.2 | −0.63 | 0.16 | 1 | 1,115 | 2,356 |
| Age | 0.08 | 0.1 | −0.11 | 0.27 | 1 | 1,359 | 2,512 |
| IQ | −0.03 | 0.11 | −0.24 | 0.19 | 1.01 | 1,046 | 2,027 |
| CBCL AP | −0.12 | 0.13 | −0.38 | 0.14 | 1 | 1,335 | 2,110 |
| Data collection wave | −0.22 | 0.25 | −0.71 | 0.27 | 1 | 1,182 | 2,415 |
| Interaction ASD × CU traits | −0.49 | 0.29 | −1.06 | 0.09 | 1.01 | 1,009 | 2,036 |
| **Interaction CD × CU traits** | **−0.66** | **0.3** | **−1.26** | **−0.07** | **1** | **1,080** | **1,951** |
| Fixations to the mouth | | | | | | | |
| **Angry faces** | **0.27** | **0.05** | **0.17** | **0.37** | **1** | **5,582** | **4,455** |
| **Fearful faces** | **0.14** | **0.05** | **0.04** | **0.25** | **1** | **5,847** | **4,293** |
| **ASD** | **0.86** | **0.42** | **0.01** | **1.67** | **1** | **1,311** | **2,308** |
| CD | 0.27 | 0.34 | −0.4 | 0.93 | 1 | 1,222 | 2,197 |
| CU traits | −0.17 | 0.21 | −0.59 | 0.24 | 1 | 1,224 | 2,306 |
| **Male sex** | **0.44** | **0.21** | **0.03** | **0.85** | **1** | **1,268** | **1,943** |
| Age | 0.02 | 0.1 | −0.18 | 0.22 | 1 | 1,627 | 2,176 |
| IQ | −0.11 | 0.11 | −0.32 | 0.1 | 1 | 1,700 | 2,446 |
| CBCL AP | −0.09 | 0.13 | −0.35 | 0.18 | 1 | 1,788 | 2,484 |
| **Data collection wave** | **0.58** | **0.26** | **0.07** | **1.11** | **1** | **1,444** | **1,902** |
| Interaction ASD × CU traits | 0.13 | 0.29 | −0.44 | 0.67 | 1 | 1,233 | 2,379 |
| Interaction CD × CU traits | 0.21 | 0.29 | −0.36 | 0.79 | 1 | 1,265 | 2,398 |

*Note*: Bold value indicates significant values. Bayes multilevel regression analysis results testing the one-sided hypotheses on key dependent variables: Number of fixations to the eyes and mouth regions. Est. = estimate or mean, Est. error = estimation standard deviations, l-95% CI = lower credible interval, u-95% CI = upper credible interval, Rhat = convergence of the MCMC algorithm (Gelman & Rubin, 1992), Bulk_ESS = bulk effective sample size estimate, Tail_ESS = tail effective sample size estimate, ASD = youth with autism spectrum disorder, CD = youth with conduct disorder, TD = typically developing youth, CBCL AP = score of the attention problems scale from the parent reported child behavior checklist.

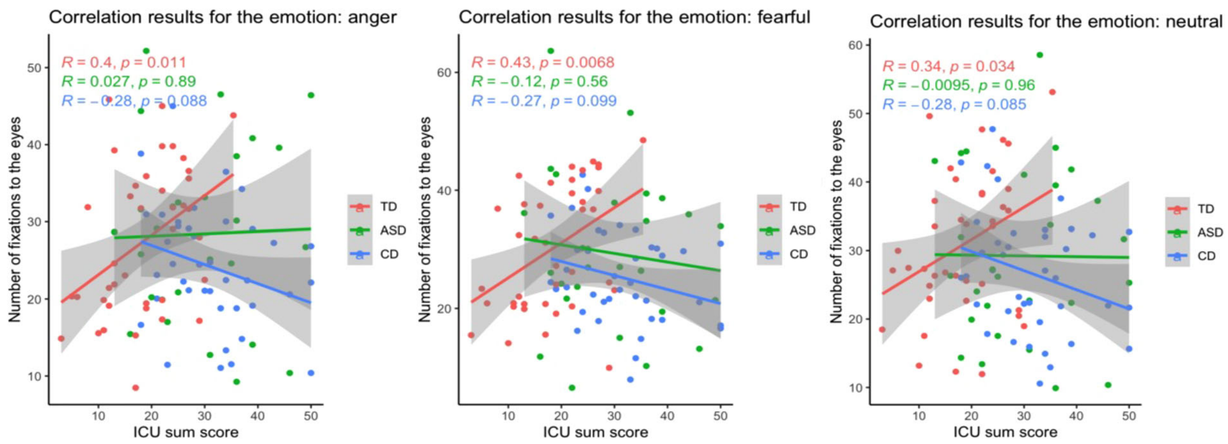


**Figure 1** Correlation analysis results of ICU sum scores and number of fixations to the eyes for each emotion per group. ASD, youth with autism spectrum disorder; CD, youth with conduct disorder; TD, typically developing youth

**Table 2** Bayes multilevel regression results – group comparisons per emotion

| | Eye fixations | | | | | Mouth fixations | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hypothesis | Est. | SE | 95% CI | | Post. prob. | Est. | SE | 95% CI | | Post. prob. |
| Anger | | | | | | | | | | |
| ASD < TD | −0.39 | 0.46 | −1.15 | 0.36 | 0.8 | 0.75 | 0.47 | −0.02 | 1.51 | 0.06 |
| CD < TD | −0.53 | 0.37 | −1.14 | 0.06 | 0.93 | 0.15 | 0.36 | −0.44 | 0.76 | 0.34 |
| ASD = CD | 0.14 | 0.32 | −0.38 | 0.65 | 0.32 | 0.6 | 0.32 | 0.07 | 1.12 | 0.03 |
| Fearful | | | | | | | | | | |
| ASD < TD | −0.3 | 0.44 | −1.03 | 0.44 | 0.75 | 1.08 | 0.45 | 0.33 | 1.81 | 0.01 |
| CD < TD | −0.58 | 0.36 | −1.18 | 0 | 0.95 | 0.4 | 0.36 | −0.18 | 1.01 | 0.13 |
| ASD = CD | 0.28 | 0.31 | −0.23 | 0.8 | 0.18 | 0.67 | 0.32 | 0.15 | 1.19 | 0.02 |
| Neutral | | | | | | | | | | |
| ASD < TD | −0.21 | 0.47 | −0.98 | 0.55 | 0.67 | 0.76 | 0.46 | 0.01 | 1.52 | 0.05 |
| CD < TD | −0.38 | 0.37 | −0.99 | 0.22 | 0.85 | 0.25 | 0.37 | −0.36 | 0.85 | 0.24 |
| ASD = CD | 0.18 | 0.32 | −0.35 | 0.71 | 0.28 | 0.5 | 0.32 | −0.02 | 1.02 | 0.05 |

Bayes regression analysis results testing the one-sided hypotheses on key dependent variables: Number of fixations to the eyes and mouth regions per emotion (anger, fearful, neutral). Est. = estimate, SE = standard error, 95% CI = credible interval, Post. Prob = posterior probability under the hypothesis against the hypothesis' alternative. Hypothesis = direction of tested hypothesis, ASD = youth with autism spectrum disorder, CD = youth with conduct disorder, TD = typically developing youth.

interaction term between group and CU traits (see Appendix S6). No main effects of CU traits and no interaction effects with group were observed.

## Discussion

The main aim of the study was to investigate the neural correlates of differences in facial emotion processing in ASD and CD groups and whether these differences can be partially explained by differences in gaze behavior. Our results showed at the behavioral level that the ASD group does not differ from CD or TD youths on eye gaze patterns but showed increased attention to the mouth region compared with TD youths. At the neural level, relative to ASD adolescents, CD youths showed reduced activation in the right anterior insula when angry faces were presented. This cluster was reduced in size but remained significant when gaze behavior parameters (number of fixations to the eye and mouth) were taken into account.

With regards to gaze patterns, our results show that eye gaze patterns did not significantly differ between youths with ASD and their typically developing counterparts; however, they show a disorder-specific increased attention to the mouth for fearful and neutral faces. These results would be in line with previous studies showing no difference in fixations on the eye region between ASD and healthy controls (Åsberg Johnels, Gillberg, Falck-Ytter, & Miniscalco, 2014; Kwon, Moore, Barnes, Cha, & Pierce, 2019). Increased attention to the mouth in fearful and neutral faces might thus be linked to deficits in facial processing, independent from the expressed emotion (Reisinger et al., 2020). Compared with the CD group, individuals in the ASD group showed increased attention to the mouth specifically when presented with negative emotions (angry, fearful), suggesting that group differences might depend on the emotional valence of the stimulus presented. Therefore, our results support the idea that individuals with ASD process facial

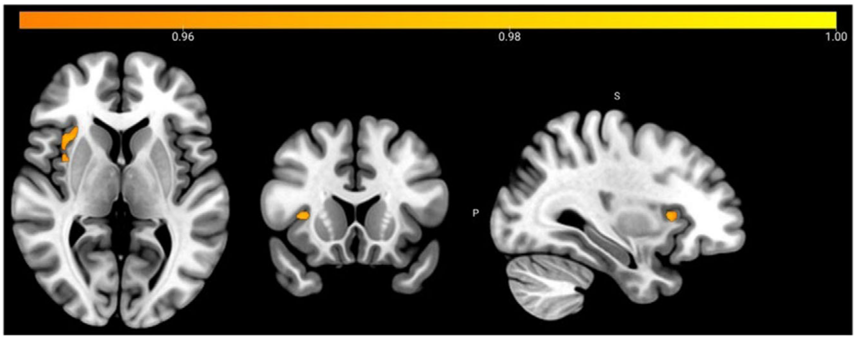


**Figure 2** Full factorial analyses for the contrast ASD > CD when angry faces are shown. The model included group as a regressor of interest and age, sex, IQ, attention problems scores of the CBCL, total sum score of the ICU, and data collection wave as regressors of no interest. The significance level is depicted in the color bar from left (0.95) to right (1). ASD, youth with autism spectrum disorder; CD, youth with conduct disorder

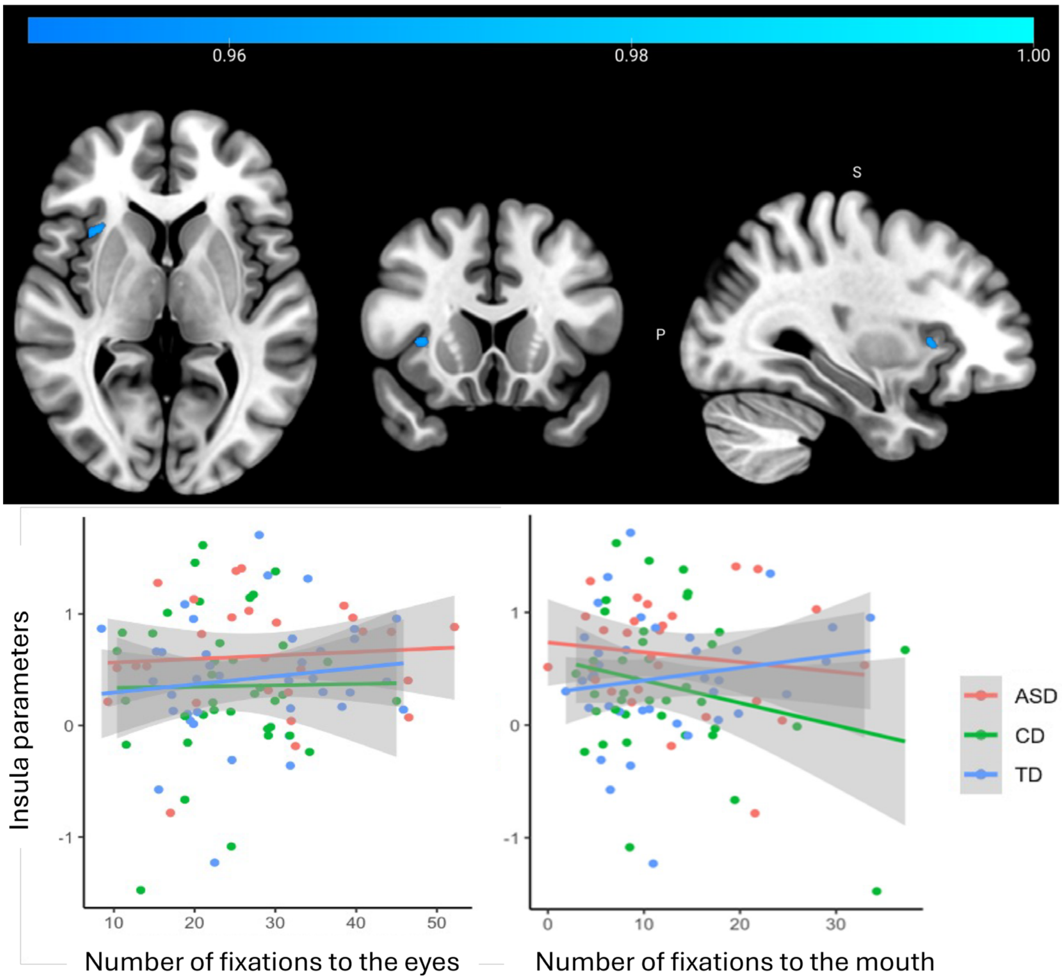


**Figure 3** Full factorial analyses results including fixations to the eye and mouth region for the contrast ASD > CD when angry faces are shown and correlation results of the number of fixations to eyes/mouth and extracted insula parameters for angry faces. The model included group and the number of eye and mouth fixations as regressors of interest and age, sex, IQ, attention problems scores of the CBCL, total sum score of the ICU, and data collection wave as regressors of no interest. The significance level is depicted in the color bar from left (0.95) to right (1). ASD, youth with autism spectrum disorder; CD, youth with conduct disorder, TD, typically developing youth

information differently not only relative to typically developing peers, but also in a way that is at least partially distinctive from youths with CD (Gross, 2005; Joseph & Tanaka, 2003). Furthermore, these results would align at least partially with the excess mouth/diminished eye gaze hypothesis of autism, which suggests the use of compensatory strategies by individuals with ASD, who would have a heightened focus on speech and its social information by fixating more on the mouth than the eye region compared to typically developing peers (Klin, Jones, Schultz, Volkmar, & Cohen, 2002).

Our findings also suggest that CU traits could be differentially associated with gaze behavior in the CD group. Although only marginally significant, the aberrant eye gaze pattern might explain to a certain extent the well-documented existence of emotion processing deficits in CD with increased CU traits (Billeci et al., 2019; Díaz-Vázquez et al., 2024). Furthermore, these findings also support existing evidence that youth with CD might pay less attention (Menks et al., 2021) or are less responsive to stimuli with negative emotional valence (Berluti et al., 2023; Fairchild et al., 2014; Marsh & Blair, 2008; Menks et al., 2021). Interestingly, while in the TD group we found a positive association between eye gaze and CU traits, participants with ASD showed no significant association between CU traits and eye gaze. Given the limited variation in CU traits in the TD group, these findings should be, however, interpreted with caution.

In sum, our behavioral findings might indicate that compared with the TD group, youth with ASD and youth with CD and high CU traits showed differences in gaze behavior. The nature of the atypical gaze pattern appears to be disorder specific. While the ASD group showed fixation abnormalities to the mouth region, CD participants with increased CU traits tended to show fewer fixations to the eye region, suggesting that atypical eye gaze in CD might be at least partially driven by co-occurring CU traits.

Brain imaging results showed no differences for the ASD or CD groups compared with the TD group. This might be potentially related to the large heterogeneity in both disorders, also in regard to emotion processing deficits (American Psychiatric Association, 2013; Kohls et al., 2020; Sivathasan, Fernandes, Burack, & Quintin, 2020; Viding & McCrory, 2019). However, our findings revealed a significant difference between youth with ASD and youth with CD. Youth with ASD displayed higher activation in the anterior insula for angry faces than those with CD. The anterior insula plays an important role in affective processes and social behavior functioning (Benarroch, 2019) and, as functional and structural abnormalities in the insula have been consistently found in youth with CD (Fairchild et al., 2019; Raschle, Menks, Fehlbaum, Tshomba, & Stadler, 2015), youth with ASD were expected to show higher insula activation compared to youth with CD. In addition, CU traits had no significant influence on brain function during emotion processing. Independent of whether CU traits were or were not included in the model, the cluster of brain activation difference between ASD and CD remained significant. Notably, inclusion of CU traits in the model increased the between-groups differences on insular activation, suggesting that these need to be taken into consideration to facilitate increased clarity on the between-group differences on neural activation picture (MacKinnon, 2000).

When mouth and eye fixations in angry facial expressions were included in the model, the size of the insula cluster was reduced, suggesting an association between gaze behavior and brain activation during facial emotion processing. This is supported by post-hoc results suggesting that differences in insula activation may be particularly linked to fixations to the mouth region in the ASD group (see Appendix S6). However, even though the cluster size was reduced when controlling for gaze behavior, the persistence of the group differences suggests that altered brain activation during facial emotion processing might go beyond the influence of gaze behavior.

This study has some limitations. Brain imaging results are tentative and thus should be interpreted with caution. It is also important to note that only those with high-functioning autism or Asperger's syndrome were recruited for intelligence comparability reasons between the groups. This subgroup's results thus do not represent the complete heterogeneity of the ASD spectrum and cannot be easily generalized. This also extends to the limited generalizability of the heterogeneity in CD. Most of our sample in the ASD and CD groups is male. Although sex is included as a covariate, it has shown to have an influence on the results (see Table 1). Therefore, further studies should investigate the potential presence of gender specificity on the neural underpinnings of deficits in facial processing. The presence of subclinical ODD and/or depressive-anxious symptoms in both disorder groups should also be considered when comparing participants with CD to those with ASD. However, the study's limited sample size restricts the ability to analyze these groups without the influence of such overlapping subclinical symptoms. Given the heterogeneity of both disorders, controlling for these overlaps could also reduce the generalizability of the findings. Additionally, the task design did not include positive emotions, and potential eye gaze differences for positive facial expressions (e.g., happy) could not be measured. Imputations were also conducted to account for the missing data in the parent-reported CBCL and CU questionnaires due to difficulties in reaching the parents or primary caregivers, especially in the CD group, of which a large proportion were placed separately from their parents.

## Conclusion

Youth with ASD did not show significant differences from the TD group in terms of fixations to the eyes but showed a disorder-specific increased attention to the mouth region compared to the TD and CD groups. Participants with CD and high CU traits showed a tendency to fixate less on the eye region of the face compared with the TD group. Although atypical gaze patterns are present in both disorders, possibly different mechanisms underpin their atypical facial emotion processing. Brain imaging results showed that gaze behavior is associated with differences in brain activation in the insula and may partially moderate brain activation differences among groups. These findings provide new insights on the emotion processing deficits observed both in ASD and CD populations. Consequently, future functional imaging studies should consider the influence of gaze behavior on disorders with atypical emotion processing such as ASD and CD. In addition, as the role of CU traits on eye gaze during emotion processing seems to be different in ASD and CD, it would be of interest to investigate in larger studies the question of whether CU traits mediate differences in eye gaze behavior. Targeting attention to salient social cues in mouth or eye regions, depending on clinical symptoms or individual risk factors such as CU traits, could potentially be beneficial in treatment interventions for disorders with facial emotion processing deficits.

## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

**Appendix S1.** Sample characteristics.

**Appendix S2.** Significance of the difference between correlations on number of fixations and ICU (inventory of callous unemotional traits) scores.

**Appendix S3.** Bayes multilevel regression results for fixation durations.

**Appendix S4.** Bayes multilevel regressions results including anxious depressive scale of the CBCL (childhood behavior checklist) as additional regressor of no interest.

**Appendix S5.** *F* test peak clusters, MNI coordinates and cluster size.

**Appendix S6.** Bayes regression results on insula parameters as outcome variable for the emotion anger.

**Figure S1.** Implicit facial emotion processing task used for the eye tracking and fMRI analysis.

**Figure S2.** Combined mask displaying the regions of interest, including areas associated with eye gaze behavior and facial emotion processing (amygdala, insula, ventromedial prefrontal cortex (vmPFC)).

**Figure S3.** Boxplots of the number of fixations to the eyes and mouth regions in percentage per group and emotion.

**Figure S4.** Boxplots of the extracted insula cluster parameter values (standardized) for each emotion per group.

**Figure S5.** Full factorial analyses for the contrast ASD > CD when angry faces are shown.

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## Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Ethical considerations

All procedures were conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee in Basel, Switzerland (EKNZ: Ethikkommission Nordwest- und Zentralschweiz, 2019-02386).

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## Key points

- Studies have shown that youth with ASD and youth with CD and high CU traits display impairments in facial emotion processing at the behavioral (reduced eye gaze) and neural (brain activation) levels, suggesting potential overlaps. The underlying neural mechanisms linked to atypical gaze behavior in ASD and CD are, however, unclear.
- This study investigated the influence of atypical gaze behavior on differences in brain activation patterns in youth with ASD, youth with CD, and typically developing peers (TD).
- Across angry, fearful, and neutral facial expressions, youth with ASD showed more fixations to the mouth region compared with youth with CD and TD. CD participants with high CU traits tended to show fewer fixations to the eye region compared with TD. Additionally, youth with ASD showed higher left anterior insula activation compared with youth with CD for angry faces. This cluster's size was reduced when controlling for the influence of gaze behavior.
- Gaze behavior partially explained brain activation differences between youth with ASD and youth with CD, highlighting gaze behavior as a potential target for treatment interventions.

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