Social communication in young children with sex chromosome trisomy: neurocognitive building blocks of behavioral outcomes

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Chapter 6

Social communication deficit in children with sex chromosome trisomy (XXY, XXX, XYY)?

Social orientation measured with eye tracking and physiological arousal responses during short communicative interaction paradigms

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Abstract

The authors studied social orientation with eye-tracking and physiological arousal responses to gain insight in how children (1-7 years) with sex chromosome trisomy (SCT) perceive and respond to communicative interactions. Assessment and recruitment took place in the USA and Western-Europe. Compared to controls (58 girls, 44 boys), children with SCT (33 XXX girls, 50 XXY boys, 24 XYY boys) showed reduced attention to the face and eyes of the on-screen interaction partner and reduced physiological arousal sensitivity in response to direct versus averted gaze. This suggest that children with SCT may experience difficulties with social communication that extend past the well-recognized risk for early language delays. These difficulties may underlie social-behavioral problems and are a promising target for early interventions.

Introduction

Due to a *de novo* error in early cell division, approximately 1:650-1:1000 children is born with an extra X or Y chromosome or sex chromosome trisomy (SCT; Berglund et al., 2019; Groth et al., 2013). An extra X chromosome leads to a 47,XXX karyotype in females or 47,XXY karyotype in males, while an extra Y chromosome in males leads to a 47,XYY karyotype. This high prevalence makes SCT one of the most common genetic disorders in humans (Hong & Reiss, 2014). SCT can be detected before birth, resulting in a relative unique opportunity to study the effects of an extra sex chromosome on neurocognitive and behavioral development from an early age. Genes that are located on both the X and Y chromosomes play an important role in neural development (Raznahan et al., 2016). Subsequently, children with SCT have an increased risk for suboptimal neurodevelopment, with studies reporting higher incidences of neurodevelopmental disorders (for a review see Van Rijn, 2019) and neurocognitive difficulties (for a review see Urbanus et al., 2019) compared to population samples.

Difficulties with language have frequently been reported in individuals with SCT. Studies on language outcomes have shown compromised language abilities in children as young as 8 months old (Zampini et al., 2020). Difficulties with language can already be noted in the preverbal stage (e.g., use of communicative gestures), and appear to cover a wide range of language abilities, including but not limited to semantic language, syntax, and pragmatic language (Bishop et al., 2011; Ross et al., 2008; Ross et al., 2009; St John et al., 2019; Urbanus, Swaab, Tartaglia, Boada, et al., 2021; Urbanus, Swaab, Tartaglia, Stumpel, et al., 2021; Zampini et al., 2020; Zampini et al., 2017; Zampini et al., 2018). As these language difficulties can already be apparent at a very young age and multiple language abilities appear to be affected, it is likely that these difficulties are anchored in early brain maturation. Considering the importance of language in social communication, it is thought that language difficulties may help explain the social behavioral difficulties that have been observed in the SCT population. However, there is more to social communication that language alone. It is important to gain more knowledge on the broader communicative skills of children with SCT. Assessments to pinpoint strengths and weaknesses in the overall communicative domain will result in knowledge that could be used for early detection of the broad spectrum of verbal and nonverbal communicative problems and ultimately for development of tailored and comprehensive intervention programs that focus on the broad spectrum of communication skills.

Preferential looking at faces and face-like stimuli over non-social stimuli is a natural phenomenon in infants and children. This preference to faces and face-like stimuli, or social attention, may facilitate communicative engagement (Frazier Norbury et al., 2009). Social attention can be divided into three constructs (Dawson et al., 2004): Social orienting (i.e., the ability to direct one's attention to another person, spontaneously or when requested; Guillon et al., 2014), joint attention (i.e., the capacity to share attention with others in a coordinated way; Nation & Penny, 2008) and attending to distress and emotions of others (i.e., the ability to understand and communicate about emotional states and desires; Sigman et al., 1992). These three constructs are crucial in early development; children with impaired social attention may experience difficulties with understanding the social world around them, which may result in compromised development of adaptive social behaviors. In addition, social attention plays an important role in language acquisition and development (Mundy & Neal, 2000). In this study, the focus will be on social orientation.

The ability to orient to the face of another individual can help children learn about speech sounds, facilitating early vocabulary learning (Hillairet de Boisferon et al., 2018). Also, the ability to orientate to relevant aspects of a social scene can reflect a child's sensitivity to pick up relevant (nonverbal) communicative cues. Focus on the mouth while looking at someone who is speaking indicates that a child scans the scene for communicative relevant information (Tenenbaum et al., 2015). In typically developing children, there is a developmental change within the social orientation to faces. This starts with a period of predominant orientation towards eyes, followed by an increased focus on the mouth during language learning, and lastly a decrease of orienting to the mouth with a simultaneous increase in looking to the eyes (Frank et al., 2012). Several studies have found associations between attention to the eyes or mouth of another person and language outcomes, both in typically developing children (e.g., Lewkowicz & Hansen-Tift, 2012; Tenenbaum et al., 2014; Tenenbaum et al., 2015), and children with neurodevelopmental disorders such as autism spectrum disorders (e.g., Habayeb et al., 2021; Stagg et al., 2014; Young et al., 2009). These studies show that these viewing behaviors are not only predictive of concurrent, but also longitudinal language outcome.

When orienting to faces, the direction of the gaze of the other person matters. Young infants already show a sensitivity to deviations in eye gaze direction, with more attention to the eyes of a person when in direct eye contact in contrast to looking away (Symons et al., 1998). This seems to differ between typically developing children and children with neurodevelopmental disorders; however, with typically developing children being more sensitive to direct gaze in contrast to averted gaze, whereas children with ASD for example, do not appear to differentiate between gaze type (Frischen et al., 2007).

Within the SCT population, only a handful of studies assessed social attention abilities in individuals with SCT. For example, in a previous study from this research group which included children from the same population, children with SCT showed reduced attention to the faces and eyes of two people engaged in a social plot and less accurate joint attention skills (Bouw et al., 2021). Studies in XXY adolescents and adults showed diminished attention to eyes while watching affective clips (Van Rijn et al., 2014) or static pictures of facial expressions (Van Rijn, 2015). In addition, adolescents and adults with XXY have a reduced tendency to focus on the eyes when presented with faces (Van Rijn, 2015). It is unknown however, if this diminished spontaneous visual attention towards social aspects in individuals with SCT is already present in early childhood and whether it is related to language outcome.

To understand and interpret individual differences in social orienting, the arousal system also needs to be taken into account. The autonomic nervous system activates and regulates this arousal system during social interactions (Porges, 2001). Arousal is necessary when responding to situational demands; modulation of arousal reflects someone's ability to attend and react in an appropriate manner to environmental demands (Roberts et al., 2008). The ability to modulate arousal differs from person to person. When someone experiences difficulties with modulating arousal levels to the situational demands, this could lead to the development of behavioral and emotional problems (Lydon et al., 2016). If someone experiences too much arousal for example, this can lead to a feeling of being overwhelmed or anxiousness, which subsequently could lead to diminished social participation. Alternatively, if someone experiences too little arousal, this could lead to less motivation to participate, resulting in a diminished focus on others during social encounters (Lydon et al., 2016). One example of a situational demand is eye contact. Eye contact or direct gaze can affect physiological arousal (Kleinke, 1986). Studies have found greater arousal responses when under direct rather than indirect gaze (for a review see Hietanen, 2018), however these responses have not yet been studied in children with SCT.

Within the SCT population, literature on arousal responses is scarce. One study showed an increased arousal response in adults with XXY when looking at emotional stimuli (Van Rijn et al., 2014). A second study used subjective arousal reports and found increased arousal to emotional events in adults with XXY (Van Rijn et al., 2006). Studies including children with SCT and studies looking at arousal in response to social communication, however, are lacking.

This study has two main aims: First, the social orientation patterns during short nonverbal communicative interactions or 'bids' will be assessed with eye tracking to answer the questions: Which information do children with SCT attend to and what information do they miss? Does gaze direction of the bid (i.e., direct/frontal gaze versus indirect/side gaze) matter? The primary focus will be on the expected difference of attention for social versus nonsocial aspects of the visual scene, and within social aspects specifically on time spent looking at the eyes and mouth of the communicative partner. We also investigated whether the development of viewing patterns towards the eyes and mouth are similar or different in the control and SCT groups and to what degree time spent looking at social aspects of the scene (i.e., the face, eyes, mouth) is associated with language outcomes, both concurrently and one year later. Second, the arousal response during the communicative bids will be assessed to determine how the autonomic nervous system responds to communicative bids, with a focus on similarities or differences in response to a direct or indirect gaze (i.e., the sensitivity to differences in gaze direction). For both aims, the SCT group will be compared to the control group, and the impact of specific SCT karyotype will be assessed as well. Lastly, several additional research questions were addressed, including if time of diagnosis, ascertainment bias, and research site played a role in explaining viewing patterns and arousal responses.

Method

Participants

The present study is part of a larger ongoing project (TRIXY Early Childhood Study) at Leiden University. The TRIXY Early Childhood Study is a longitudinal study that aims to identify neurodevelopmental risk in young children with an extra X or Y chromosome. Within the present study, children aged 1-7 years at enrollment were included.

Recruitment took place in the Netherlands, Belgium, and Colorado USA. Children with SCT were recruited with the help of clinical genetic departments, pediatricians, and national support and advocacy groups. Children in the control group were recruited with help of public institutions (e.g., public daycare centers and primary schools) and via the civil registry. Recruitment of the control group took place in the western parts of the Netherlands. Assessments took place at the Trisomy of the X and Y (TRIXY) Expert Center the Netherlands and the eXtraordinarY Kids Clinic in Developmental Pediatrics at Children's Hospital Colorado.

For both the SCT as well as the control group, the following exclusion criteria applied: A history of traumatic brain injury, severely impaired hearing or sight, neurological illness, or colorblindness. Specific for the control group, children with a previous diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association,

2013) were excluded. In addition, as inclusion criterion for both groups, both the child and the (primary) parent/caregiver had to speak Dutch or English. All children had normal or corrected-to-normal vision. Specific for the SCT group, children were included if the trisomy was present in at least 80% of the cells, as confirmed by standard karyotyping. Due to ethical reasons, genetic screening was not performed in the control group. However, based on the prevalence of SCT, the risk of including a child with SCT in the control group was considered minimal and acceptable.

In total 107 children with SCT (33 XXX, 50 XXY, 24 XYY) and 102 controls (58 XX, 44 XY) were included. Ages ranged from 1.00-7.66; years, mean age did not differ between the SCT (M = 3.68, SD = 1.94) and control group (M = 3.61, SD = 1.62; p = .751). Global intellectual functioning was assessed with the Bayley Scales of Infant and Toddler Development (third edition; Bayley, 2006), the Wechsler Preschool and Primary Scale of Intelligence (third edition; Wechsler, 2002) or the Wechsler Nonverbal Scale of Ability (Wechsler & Naglieri, 2006). On average, global intellectual functioning was lower in the SCT (M = 96.58, SD = 17.63) than the control group (M = 105.70, SD = 14.34; $p < .001$). As a proxy for social economic status (SES), parents were asked to report the highest level of completed education. If two caregivers were present (96.2%), SES was computed as the average of both caregivers. The Hollingshead criteria were used to account for differences in educational systems between countries (Hollingshead, 1975). On average, SES was higher in the SCT group (M = 5.92, SD = .94), than in the control group (M = 5.43, SD = 1.40; p = .003). Children recruited in the USA where White (88.1%), Black or African American (3.4%) or Asian (3.4%) or 'unknown' (5.1%). Information on race/ethnicity in the sample recruited in Western-Europe was not available. Descriptive statistics for age, global intellectual functioning, and SES between the SCT and control group and between the SCT karyotypes can be found in Table 1.

Table 1. Descriptives SCT versus control and SCT karyotypes

	SCT	**Control**	***p* (SCT vs Control)**	**XXX**	**XXY**	**XYY**	**SCT comparisons[d]**
N	107	102		33	50	24	
Age (years)	3.65 (1.91)	3.61 (1.62)	.865	4.26 (1.74)	3.25 (1.93)	3.80 (2.05)	.062
GIF[a]	96.58 (17.63)	105.70 (14.34)	$<$.001	94.69 (16.33)	99.48 (17.73)	92.86 (19.00)	.275
SES[b]	5.92 (.94)	5.43 (1.40)	.003	5.91 (1.03)	6.06 (.88)	5.67 (.90)	.239
Ascertainment Bias[c] (A/B/C)				11/12/10	28/15/7	16/3/5	.063
Time of Diagnosis (Prenatal/Postnatal)				20/13	35/15	16/8	.675

Note: scores represent Means (SD)
[a] Data for 6 children with SCT was incomplete (1 XXX, 2 XXY, 3 XYY)
[b] Data for 1 child with SCT was not available
[c] A = Active prospective follow-up; B = Information seeking parents; C = Clinically referred cases
[d] SCT comparisons: XXX versus XXY versus XYY
Abbreviations: GIF = global intellectual functioning / IQ; SES = social economic status

Within the SCT group 71 children received a prenatal diagnosis of SCT as a result of prenatal screening or screening for example due to advanced maternal age. Children that received a postnatal diagnosis (N = 36) received a diagnosis of SCT due to a developmental delay (N = 15), physical and/or growth problems (N = 12) or medical concerns (N = 9). In addition to time of diagnosis, the reason families enrolled in the study was monitored (i.e., ascertainment bias). Three subgroups were identified: 1) 'Active prospective follow-up' (51.4%

of the SCT group), 2) 'Information seeking parents' (28.0% of the SCT group), and 3) 'Clinically referred cases' (20.6%) of the SCT group. Distributions in time of diagnosis and ascertainment bias were similar between the three SCT karyotypes (see Table 1.).

Instruments

Communicative Bids Paradigm

The paradigm consisted of two dynamic video clips of 30 seconds each. In both video clips children were shown a scene of naturalistic caregiver interaction; a female actress smiling and trying to engage using simple universal sounds (e.g., 'hi', 'oh'). The use of language during a communicative bid might be a confounding factor, where children do not necessarily attend to social aspects of a scene naturally, but rather attend to the eyes or mouth of the communicator as a response to hearing language (Brooks & Meltzoff, 2005). For that reason, no speech other than simple sounds were used in the paradigm of this study. Two objects were positioned on the left and right of the actress. In the first video clip, the actress looked directly at the child (frontal gaze direction), whereas in the second video clip the actress was facing sideways – looking towards a point at the right of the child (side gaze direction). Dynamic video clips were used, as the ecological validity is higher for dynamic video clips rather than static pictures. See Figure 1 for a still of the dynamic video clips.

Figure 1. Communicative bids paradigm: Frontal gaze direction (left) and side gaze direction (right)

Eye Tracking: Apparatus

Eye gaze data was collected with a Tobii X2-60 eye tracking device, which records the X and Y coordinates of the position of the eye using a corneal reflection technique (Tobii Technology AB, Danderyd, Sweden). Stimuli were shown on a 15.6-inch laptop with a resolution of 1920x1080 pixels. A sampling frequency of 60 Hz was used.

Eye Tracking: Processing Procedure

Gaze data was processed with Tobii studio version 3.4.8. The Tobii-IV fixation filter was used for defining visual fixations (Olsen, 2012). Areas of interest (AOI) included the total screen, objects, and face, eyes, and mouth of the actress and were drawn with the 'dynamic AOI' tool in Tobii studio. An extended region of 1 cm surrounding the AOI was included to create sufficiently large AOI, as large AOI are more robust to noise (Hessels et al., 2016). There was no overlap between AOI. Both total visit duration and total fixation for the AOI were assessed.

Physiology: Apparatus

Heartrate was used as an indicator for arousal levels. Heartrate data was collected AcqKnowledge (version 5.0.2; BIOPAC Systems Inc.). Recordings were acquired with an Electrocardiogram amplifier (ECG100C) and a BIOPAC data acquisition system (MP150

Windows) at a sampling rate of 1000 Hz. Heart rate was recorded simultaneously with the eye tracking data. The physiological equipment was synchronized with the Tobii software with markers representing the start of the video clips.

Physiology: Processing Procedure

Heartrate data was processed with PhysioData Toolbox v0.5 (Sjak-Shie, 2019). Recorded data was manually inspected by detecting R peaks. With visual identifications, motion artifacts were identified and excluded from the data.

Receptive and Expressive Semantic Language Skills

One-year-olds

In the one-year-old children, semantic language skills were assessed with the Bayley Scales of Infant and Toddler Development – Language scale (Bayley, 2006). This scale consists of separate subtest for receptive and expressive semantic skills. In the receptive subtest, depending on the age of the child, pre-verbal behavior, ability to identify objects and pictures, and understanding of verbal messages was assessed. In the expressive subtest, depending on the age of the child, pre-verbal communication and the ability to name objects and pictures was assessed.

Three-to-seven-year-olds

In children aged 3 years and older, receptive semantic skills were assessed with the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997, 2005). The PPVT assesses the child's listening comprehension to spoken words, where the child must identify the picture (out of 4 pictures) that is orally presented by the researcher. Expressive semantic skills were assessed with the Clinical Evaluation of Language Fundamentals Preschool edition (CELF-P; Wiig et al., 2004, 2012). The CELF-P assesses the child's ability to label people, objects, and actions based on colored images.

Procedure

This study was approved by the Ethical committee of Leiden University Medical Center, the Netherlands, and the Colorado Multiple Institutional Review Board (COMIRB) in Colorado, USA. Written informed consent according to the declaration of Helsinki was obtained after providing a description of the study to the parent(s) of the child.

Assessments took place in either a quiet room at the university or at home. As assessments took place at various sites (the Netherlands, Belgium, Colorado USA), the test set-up and research protocol were identical on all sites. Researchers from the Dutch site were responsible for project and data management (i.e., training and supervision of researchers, processing and scoring of data).

Language assessments were administered in either Dutch or English. All tests were administered according to the standardized procedure as specified in the instrument's manual. Neurocognitive assessments, including assessment of receptive and expressive language took place before the eye tracking and physiology assessments to get the child acquainted with the examiner and testing location. For the eye tracking and physiology assessments, the laptop with the eye tracker was placed on an adaptable table to adjust to the height of the child. The table was placed in a small tent to minimize diversions. The child was seated in a comfortable car seat at approximately 65-centimeter viewing distance. Recording electrodes were placed on the child in the presence of the parent. To familiarize the child with the electrodes, and for the electrode to properly attach to the skin, the child watched a movie on the laptop for 5-10 minutes before the eye tracking and physiological recording. One electrode was placed 10 cm below the suprasternal notch, a second electrode was placed 10 cm above the bottom of the rib cage on the right side of the child. A ground electrode was included by simultaneously recording electrodermal activity (not included in the current study).

Before the paradigm was shown, a 5-point calibration procedure was conducted. The video clips were shown in a fixed order, all preceded by an attention grabber (i.e., a moving picture of an animal shown on a black background, accompanied by a sound). First, a three-minute resting clip was shown to assess baseline arousal levels. During this time, children looked at fish in an aquarium. Next, the frontal video clip was shown, followed by a 30 second resting clip showing a ball and a slide. Finally, the side video clip was shown. The researcher sat on the left of the child and controlled the Tobii via a remote keyboard. A second researcher controlled the BIOPAC. All physiology equipment was placed outside the sight of the child. Children were instructed to sit quietly and watch the video clips.

Study Design

The TRIXY early childhood study is a longitudinal study with an initial baseline and a one-year follow-up assessment. Within the present study, eye tracking data and arousal assessments during the communicative bids paradigm from the initial assessment were reported for both the SCT and control group. For language assessments, outcomes from the initial assessment as well as the follow-up assessment were reported for the SCT group only. Follow-up language outcomes in combination with valid baseline eye tracking data was available only for a subset of the SCT group ($N = 47$), with follow-up assessments taking place 47-61 weeks after initial assessment ($M = 53$, $SD = 2.64$). The high number of missing data is largely due to the worldwide COVID-19 pandemic, where families were unable to participate and/or assessments had to be postponed (i.e., took place > 18 months after baseline; $N = 26$). Other reasons included invalid eye tracking data ($N = 23$), or families were unable to schedule visits due to family circumstances ($N = 6$).

Statistical Analyses

For the eye tracking data, variables were computed to represent the proportion of time children looked at each AOI. First, for the frontal and side clips separately, the attention to the screen was calculated by dividing each child's total visit duration to the screen by 30 (i.e., the duration of the clip) and multiplied by 100. Next, the percentage of time a child fixated to the objects, face, eyes, and mouth was calculated by dividing the total fixation duration for the AOI by the proportion of time the child attended to the screen and multiplied by 100. Main interests in this study were the total time children attended to the screen, the time children spent looking at social versus nonsocial aspects of the scene, and the time children spent looking at the eyes versus the mouth of the actress.

For the physiological data, the first 30 seconds of the baseline clip were considered as 'baseline arousal level'. Heartrate data that was collected during the social attention bids eye tracking paradigm was summarized in 10 second epochs. Delta scores (Δ) were computed by subtracting the baseline arousal level from the heartrate for each epoch.

Several parametric and non-parametric tests were used. First, to compare SCT versus control children, independent samples t-tests or Repeated Measures MANOVA were used to compare outcomes. If there was unequal variance-covariance (i.e., Box's M $p < .05$), Pillai's trace was used to interpret the effect. Significant interaction effects were followed by within-group paired samples t-tests. Second, to assess the impact of SCT characteristics, non-parametric Kruksal Wallis tests or MAN(C)OVA were used, depending on sample sizes and comparability of age between groups. Second, to assess the effect of age on outcomes PROCESS moderation analyses were used (Hayes, 2017). The interaction effect between research group and either time spent looking at the eyes or mouth or arousal levels was examined and if applicable followed up with correlations for the SCT and control group separately. Lastly, correlations were calculated between eye tracking outcomes and both concurrent and future language outcomes in children with SCT.

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) Version 25. Level of significance was set at $p \leq .05$. Effect sizes were calculated with partial η^2 and interpreted according to the guidelines by Cohen (1988), with partial η^2 .01 considered as small, partial η^2 .06 as medium, and partial η^2 .14 as large.

Results

Eye Tracking: Communicative Bids - Preliminary Analyses

Data for the eye tracking paradigm was missing for 20 children (9.6%; $N_{SCT} = 16$), due to technical issues or fatigue of the child. As an indication for the reliability of the data, the total proportion of time children spent looking at the screen (for the frontal and side gaze direction separately) was screened for children who did not contribute sufficient data (30% or 10 seconds). For 15 children (7.2%; $N_{SCT} = 10$) the data for one or both of the gaze directions was deemed insufficient, and these children were discarded from the analyses. After exclusion of these children, Z-scores were calculated for each of the AOI of interest for the SCT and control group separately. Depending on the analysis, a filter was used to select children with appropriate Z scores between -3 and 3 (i.e., for the Social vs Objects analysis only children with $-3 > Z < 3$ for the AOI face and objects for both the frontal and side gaze direction were included).

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Overall, 174 children successfully completed the eye tracking paradigm with reliable data (81 SCT and 93 controls). As an indication of overall attention to the paradigm, attention to the screen collapsed for frontal/side gaze direction was used. On average, children attended to the video 90.4% of the time the videos were displayed. An independent samples t-tests indicated similar attention to the screen between the SCT (89.4%) and control group (91.3%, $p = .245$).

As there were significant differences in IQ and SES between the SCT and control group, correlations were calculated between these variables and three global eye tracking outcome measures (screen, face, objects collapsed for frontal/side gaze direction). No significant correlations were found (see supplementary materials, Table A.), therefore, IQ and SES were not included in further analyses regarding eye tracking outcomes.

Eye Tracking: Communicative Bids - Attention to Social versus Nonsocial Information

In total, 78 children in the SCT group and 89 children in the control group were included in the social versus nonsocial analysis. The proportion of time children spent looking at social (i.e., the face of the actress) versus nonsocial (i.e., objects on the sides of the actress) aspects of the scene was analyzed for the factor 'gaze direction' (frontal versus side), with research group (SCT versus control) as a between subjects variable. The Repeated Measures MANOVA showed a significant main multivariate effect of research group, Wilks' Lambda = .95, $F(2,164)$

= 4.46, p = .020, partial η^2 = .05 and a significant main multivariate effect of gaze direction, Wilks' Lambda = .95, F(2,164) = 4.27, p = .016, partial η^2 = .05. The interaction effect of Research group x gaze direction was not significant, Wilks' Lambda = .99, F(2,164) = .80, p = .451, partial η^2 = .01. The significant main effects were further analyzed with univariate tests.

Regarding the main effect of gaze direction (frontal versus side), univariate tests for objects showed that children, regardless of research group, spent proportionally more time looking at the objects in the frontal gaze direction (EMM = 11.52, SE = .73) compared to the side gaze direction (EMM = 9.71, SE = .58), p = .012. Partial η^2 = .04, indicating a small effect. No differences between gaze direction were found for time spent looking at the face, p = .511. Regarding the main effect of research group, results showed that, regardless of gaze direction, children with SCT spent proportionally less time looking at the face (EMM = 47.39, SE = 2.10) than children in the control group (EMM = 55.27, SE = 1.96), p = .007, partial η^2 = .04, indicating a small effect. No significant differences between children with SCT and the control group were found for time spent looking at objects (p = .362).

To evaluate if significant deviations in the SCT group in terms of overall looking time towards the face (irrespective of gaze direction) was impacted by specific SCT karyotype, a nonparametric Kruskal Wallis test was used for a more in-depth analysis within the SCT group. No significant subgroup effects were found (p = .090); indicating that there were no significant differences in attention to faces between the three karyotypes (XXX, XXY, XYY).

Taken together, these results indicate that both children with SCT and controls do not seem to differentiate between gaze direction (frontal/side) when looking at a face, but children in both groups do tend to look more at objects during a direct (frontal) compared to an indirect (side) communicative bid. In addition, compared to controls, children with SCT are less inclined to fixate on the face during a communicative bid, but attend equally towards nonsocial objects. This diminished attention to the face appears to be irrespective of SCT karyotype.

Eye Tracking: Communicative Bids - Eyes versus Mouth

In total, 77 children in the SCT group and 91 children in the control group were included in the eyes versus mouth analysis. The proportion of time children spent looking at the eyes versus the mouth was analyzed for the two gaze directions (frontal versus side), with research group (SCT versus control) as a between subjects variable. The Repeated Measures MANOVA showed a significant main multivariate effect of research group, Pillai's Trace = .04, F(2,165) = 3.79, p = .025, partial η^2 = .04 and a significant main multivariate effect of gaze direction, Pillai's Trace = .10, F(2,165) = 9.12, p < .001, partial η^2 = .10. The interaction effect of Research group x gaze direction was not significant, Pillai's Trace = .01, F(2,165) = .63, p = .537, partial η^2 = .01. The significant main effects were further analyzed with univariate tests.

Regarding the main effect of gaze direction (frontal versus side), univariate tests for attention to the mouth showed that children, regardless of research group, spent proportionally more time looking at the mouth of the actress in the frontal gaze direction (EMM = 16.13, SE = 1.18) compared to the side gaze direction (EMM = 13.13, SE = 1.10), p < .001. Partial η^2 = .07, indicating a moderate effect. There was no effect of gaze direction on time spent looking at the eyes of the actress, p = .110. Regarding the main effect of research group, results showed that, regardless of gaze direction, children with SCT spent proportionally less time looking at the eyes (EMM = 18.40, SE = 1.95) than children in the control group (EMM = 23.94, SE = 1.79), p = .038. Partial η^2 = .03, indicating a small effect. No significant differences between children with SCT and the control group were found for time spent looking at the mouth of the actress (p = .418).

To evaluate if significant deviations in the SCT group in terms of overall looking time towards the eyes (irrespective of gaze direction) was impacted by specific SCT karyotype, a nonparametric Kruskal Wallis test was used for a more in-depth analysis within the SCT group. No significant differences were found (p = .596); indicating that there were no significant differences in attention to eyes between the three karyotypes (XXX, XYY, XYY).

Taken together, these results indicate that both children with SCT and controls do not differentiate between gaze direction (frontal/side) when looking at eyes, but they do tend to look more at the mouth during a direct compared to an indirect communicative bid. In addition, compared to controls, children with SCT are less inclined to fixate on the eyes during communicative bids, but attend equally to the mouth. This diminished attention to the eyes appears to be irrespective of SCT karyotype.

Eye Tracking: Communicative Bids - Effect of Age

The effect of age on fixation to the eyes and mouth was explored with Process analyses and followed up with correlations. As previous analyses showed no significant research group x gaze direction interactions for the mouth nor the eyes, the frontal and side gaze direction were collapsed as an indication for the overall fixation to the mouth or eyes.

First, for time spent looking at the eyes, age by group interactions were explored with a PROCESS analysis with time spent looking at the eyes as dependent variable, research group as independent variable, and age as moderator. Group effects were not significantly moderated by age, t = .57, p = .570. This indicates that differences between the children with SCT and controls in time spent looking at the eyes were stable across ages. To further examine this relationship, correlations between time spent looking at the eyes and age were calculated for the SCT and control group separately. In both the control and SCT group, there were weak but significant correlations between age and time spent looking at the eyes (controls: r = .21, p = .051; SCT: r = .23, p = .047). In other words, for both children with SCT and controls, children spent more time looking at the eyes when age increased. A visualization of these results can be found in Figure 2.

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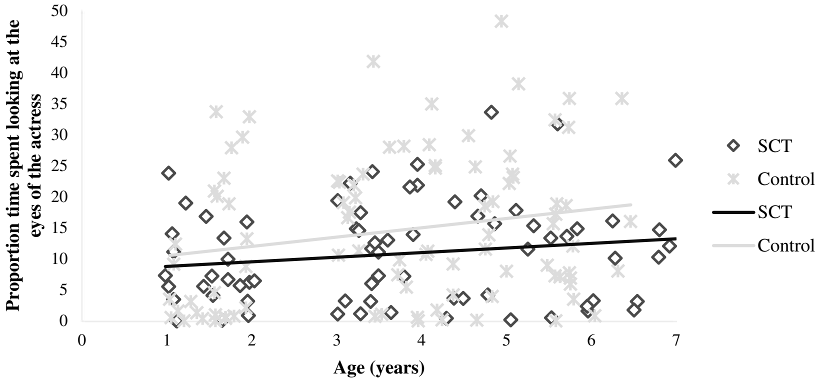

Figure 2. Proportion time attended to the eyes in the SCT and control group at different ages

Second, for time spent looking at the mouth, age by group interactions were explored with a PROCESS analysis with time spent looking at the mouth as dependent variable, research group as independent variable, and age as moderator. Group effects were not significantly moderated by age, $t = -.87$, $p = .384$. This indicates that differences between the children with SCT and controls in time spent looking at the mouth were stable across ages. To further examine this relationship, correlations between time spent looking at the mouth and age were calculated for the SCT and control group separately. In the control group, there was a weak but significant relation between age and time spent looking at the mouth, $r = -.22$, $p = .039$; when age increased, children in the control group attended less to the mouth. The relationship between age and time spent looking at the mouth in the SCT group however, failed to reach significance, $r = -.15$, $p = .181$. This indicates that although time spent looking at the mouth might decrease in the SCT group as well, this decrease was not statistically significant. A visualization of these results can be found in Figure 3.

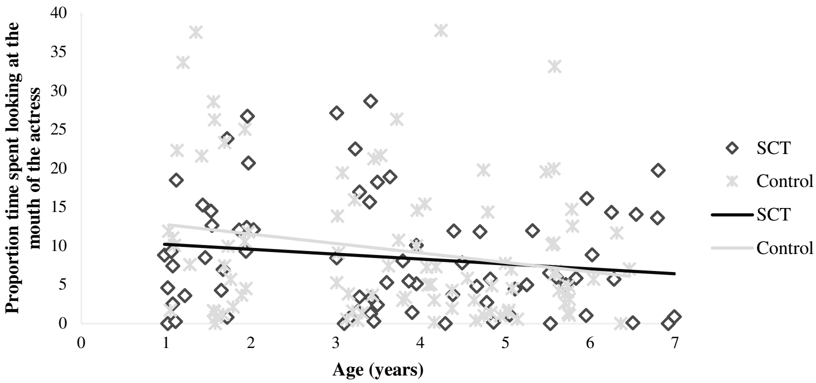

Figure 3. Proportion time attended to the mouth in the SCT and control group at different ages

Attention to the Eyes and Mouth: Correlations with Language Outcomes

Within the SCT group, correlations were calculated between the proportion children spent looking at an AOI (face, eyes, mouth) and both concurrent and future language skills (i.e., at one-year follow-up). To control for age effects, the SCT group was split into three age groups: 1-year-olds, 3-4-year-olds, and 5-7-year-olds. These age groups were comparable in the distribution of karyotypes ($p_{concurrent} = .155$; $p_{future} = .262$) and ascertainment bias ($p_{concurrent} = .281$; $p_{future} = .514$). Regarding time of diagnosis, there was a difference in the distribution between the age groups when including concurrent language outcomes ($p = .011$), with more prenatal diagnoses in the younger age group, but there was no difference in the distribution of time of diagnosis between the age groups when including future language outcomes ($p = .080$).

For the 1-year-olds with SCT, significant correlations were found for looking at the eyes and mouth and both concurrent and future language skills. More specifically, in one-year old children with SCT, children who attended more to the mouth of the actress had significantly better concurrent and future receptive and expressive semantic skills. Simultaneously, time spent looking at the eyes of the actress was significantly negatively correlated with concurrent expressive semantic skills, and future receptive and semantic skills. For the 3–4-year-old children with SCT, no significant relations were found between time spent looking at the eyes or mouth and concurrent or future language skills. In the children with SCT aged 5-years and older, a trend was found between time spent looking at the mouth and future receptive semantic skills; although not significant, this could indicate that looking at the mouth could be associated with better receptive semantic skills at the follow-up assessment. Correlations per age-group can be found in Table 2.

Table 2. Correlations between current (c) and future (f) language (1-year follow-up) in children with SCT

	1-year-olds			**3-4-year-olds**			**5-7-year-olds**		
	N_c = 22 / N_f = 16			**N_c = 28 / N_f = 16**			**N_c = 22/23 / N_f = 15**		
	Face	Eyes	Mouth	Face	Eyes	Mouth	Face	Eyes	Mouth
Current receptive semantic skills	.19	-.36	.66***	.16	.14	.08	.01	-.15	.18
Current expressive semantic skills	.21	-.45*	.65**	-.12	.05	-.17	.26	.33	.19
Future receptive semantic skills	.09	-.66**	.59**	-.02	.24	-.26	.28	.22	.43[a]
Future expressive semantic skills	.26	-.71**	.74**	-.05	.16	-.02	.19	.22	.03

* $p < .05$; ** $p < .01$; *** $p < .001$ (one-sided)
[a] Trend towards significance, $p = .055$

Arousal Response for Different Gaze Directions: Preliminary Analyses

Children who had missing data or a low reliability on the eye tracking measures were excluded in the arousal analyses as well (16.8%). In addition, children with unreliable physiology data, for example due to a large amount of motion artifacts or malfunctioning hardware, or children who had no (reliable) baseline data were excluded (9.6%). After exclusion of this data, Z-scores were requested for the six 10 second epochs for the SCT and control group separately. A filter was used to select data, only including children with Z scores between -3 and 3 in the analysis.

Overall, 149 children with reliable data were included in the arousal analyses (65 children with SCT and 84 controls). There was no significant difference in average baseline heartrate between the SCT group (M = 102.27, SD = 16.22) and control group (M = 101.93, SD = 13.92). There were, however, significant differences between the three SCT karyotypes, with higher baseline HR in the XXY compared to the XXX and XYY group, and the latter not significantly different. To account for individual within-group differences in baseline heartrate, delta (Δ) scores were computed by subtracting the baseline heartrate from the heartrate for each epoch.

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First, to evaluate the effectivity of the paradigm in triggering the arousal system, the effect of the frontal and side gaze directions over time was assessed in the control group only. A Repeated Measures ANOVA with gaze direction (frontal versus side) and time (Δ-scores in 3 epochs) revealed a significant interaction effect between gaze direction and time ($p < .001$). Paired samples t-test per epoch (e.g., frontal 1 vs side 1) revealed that the arousal response in the control group differed between the frontal and side gaze direction in the first epoch (10 seconds, $p < .001$), but not in the remaining epochs (p ranging from .332 - .475). This illustrates that children in the control group had a different initial arousal response to the frontal versus side gaze direction; in other words, there was a sensitivity for gaze direction in the first stages of communication. To assess the arousal response in children with SCT in this sensitive time window, only Δ-scores in the first epochs for the frontal and the side gaze direction were included in subsequent analyses.

As there were significant differences in IQ and SES between the SCT and control group, correlations were calculated within the SCT group between these outcomes and Δ-arousal scores for the initial 10 seconds (frontal and side gaze) to assess if arousal levels were dependent on IQ or SES. No significant correlations were found (see supplementary materials, Table A.), therefore, IQ and SES were not included in further analyses regarding physiological outcomes.

Arousal Response for Different Gaze Directions: SCT versus Controls

In total, 65 children in the SCT group and 84 children in the control group were included in the analysis. The Δ-arousal levels within the two gaze directions (frontal versus side) were included as within subjects variable with research group (SCT versus control) as a between subjects variable. The Repeated Measures MANOVA showed a significant research group x gaze direction interaction effect, Wilks' Lambda = .96, $F(1,147) = 5.89$, $p = .016$, partial $\eta^2 = .04$.

The significant interaction effect was further explored with post-hoc paired-samples t-tests. Whereas children in the control group had a different initial response to the frontal versus side gaze direction: A stronger response to the side (ΔHR = -4.94, *SD* = 5.34) compared to the frontal gaze direction (ΔHR = -1.71, *SD* = 6.31), a different pattern was found in the SCT group. In the SCT group, the paired-samples t-test of the did not indicate a difference in arousal response to the gaze direction, $t(64) = 1.09$, $p = .281$, with similar responses in the frontal (ΔHR = -2.50, *SD* = 6.61) and the side gaze direction (ΔHR = -3.26, *SD* = 5.14). A visualization can be found in Figure 4.

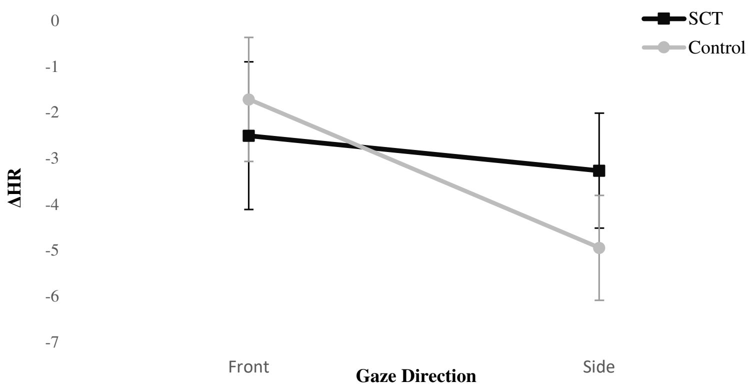

Figure 4. Sensitivity to direction of eye gaze in the SCT and control group

To evaluate if significant deviations in the SCT group in terms of this reduced arousal sensitivity was impacted by specific SCT karyotype, a nonparametric Kruskal Wallis test was used for a more in-depth analysis within the SCT group. A $\Delta HR_{sensitivity}$ score was calculated by subtracting the ΔHR in the side gaze direction from the ΔHR in the frontal gaze direction (first epoch only), where a higher score indicates more sensitivity to gaze direction. With a nonparametric Kruskal Wallis test, gaze direction sensitivity was compared between the three karyotypes (XXX, XXY, XYY). No significant differences were found ($p = .869$) indicating that there are no differences in sensitivity to gaze direction between the three karyotypes.

Collectively, these results indicate that compared to controls, the arousal system of children with SCT appears to be less sensitive to gaze direction. These findings appear to be irrespective of SCT karyotype.

Arousal Response for Different Gaze Directions: Effect of Age

The effect of age on sensitivity to differences in gaze direction was explored with a Process analysis and followed up with correlations. For these analyses $\Delta HR_{sensitivity}$ was used as an indication of gaze direction sensitivity, with higher scores indicating more sensitivity.

A PROCESS analysis with arousal sensitivity as dependent variable, research group as independent variable, and age as moderator showed that group effects were not significantly moderated by age, $t = .1.61$, $p = .110$. This indicates that the pattern of sensitivity to gaze direction across ages is not statistically different for children with SCT and controls. To further examine this relationship, correlations between arousal sensitivity and age were calculated for the SCT and control group separately. No significant correlations between age and sensitivity to gaze direction were found in either the control ($r = .12$, $p = .290$) or SCT group ($r = -.16$, $p = .215$). In other words, for both children with SCT and controls, gaze direction sensitivity (i.e., arousal level) was relatively stable across the age range. A visualization of these results can be found in Figure 5.

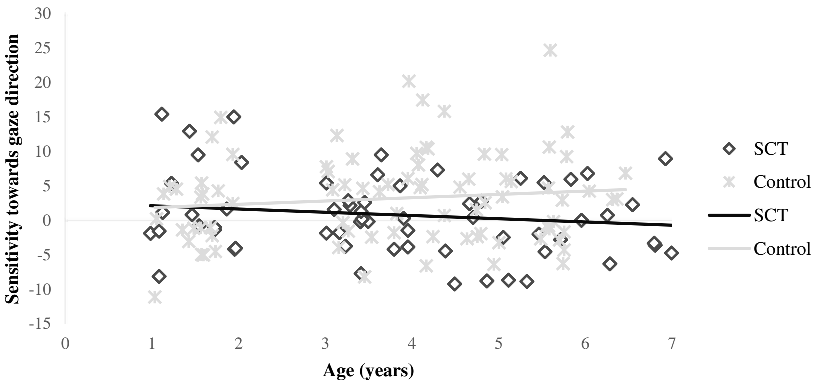

Figure 5. Arousal sensitivity towards gaze direction in the SCT and control group at different ages

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Impact of SCT Characteristics on Social Orientation and Arousal Sensitivity

Additional analyses within the SCT group were done to assess to what degree differences between the SCT and control group were impacted by characteristics of the SCT group (i.e., time of diagnosis, ascertainment bias, research site). For the eye tracking outcomes, time spent looking at the face and eyes was explored further (collapsed for gaze direction), for arousal outcomes, sensitivity to gaze direction was explored further. All outcomes can be found in the supplementary materials (Table B.). When comparing SCT subgroups, these subgroups did not differ in distribution of karyotypes, time of diagnosis, and ascertainment bias (when applicable). When comparing subgroups that differed in age, age was included as covariate in the analysis.

Regarding time spent looking at the face, no differences were found between children with a prenatal versus postnatal diagnosis ($p = .881$), for children who were actively followed-up, information seeking, or clinically referred (i.e., ascertainment bias; $p = .821$), or for children from the USA versus EU site ($p = .262$). Regarding time spent looking at the eyes, no differences were found between children with a prenatal versus postnatal diagnosis ($p = .248$), for children who were actively followed-up, information seeking, or clinically referred (i.e., ascertainment bias; $p = .432$), or for children from the USA versus EU site ($p = .117$). Lastly, regarding sensitivity to gaze direction, no differences were found between children with a prenatal versus postnatal diagnosis ($p = .512$), for children who were actively followed-up, information seeking, or clinically referred (i.e., ascertainment bias; $p = .073$), or for children from the USA versus EU site ($p = .491$).

Discussion

This study aimed to increase knowledge of how young children with SCT respond to short periods of communicative interactions (i.e., communicative ‘bids’). Overall, this study shows that children with SCT appear to attend less to the face, and specifically the eyes of another person during communicative bids. In very young children (1-year-olds), social orientation was strongly correlated to both concurrent and future language outcomes at one year follow-up. In addition, the arousal system of children with SCT appears to be less sensitive to differences in gaze directions.

This study used a dynamic eye tracking paradigm, with an actress that smiles and uses simple sounds rather than speech to study responses to communicative bids in an ecologically valid way. Previous studies have shown that language and communicative development are among the most affected neurocognitive outcomes in individuals with SCT (e.g., Boada et al., 2009; Urbanus et al., 2019). It is possible that diminished social attention already present very early in life plays a significant role in this. This study shows that young children with SCT orient less to social aspects during communicative interactions (i.e., the face). However, this does not seem to be due to increased attention towards objects. Further exploring this reduced attention to social aspects showed that children with SCT orient less to the eyes of another person, however orientation to the mouth did not differ from controls. This is particularly striking, as attention to the mouth is believed to be adaptive for language learning, and it could be expected that children with SCT, for whom language is a vulnerable domain, would show deviances in looking towards the mouth. Social orientation was modulated by gaze direction in a similar way to the control group; in other words, children with SCT do not appear to differ in sensitivity to the direction of eye gaze while watching a social scene such as a communicative bid. Taken together, it appears that children with SCT experience difficulties orienting towards social aspects of a scene. It is possible that this reduced attention plays a role in picking up social signs, that are important for adequate communicative competence. Sensitivity to these social signs, such as eye tracking, is important as it may lead to a heightened receptive state for upcoming information (Csibra & Gergely, 2009) and to a better understanding of for example another person’s mental state (Farroni et al., 2002). In other words, the ability to orient to social aspects of a social scene facilitates neurocognitive development. As some children with SCT appear to have difficulties with attending to social cues, this could play a role in the increased risk for neurocognitive and neurobehavioral difficulties that are reported in this population (e.g., Urbanus et al., 2019; Van Rijn, 2019).

When looking at the arousal system, and more specifically to evaluate if children with SCT are able to adapt to situational demands (i.e., direct versus indirect gaze), we observed a different pattern compared to the control group. In the control group, the level of arousal was dependent on direction of gaze during the communicative bids, in other words, children in the control group modulated their arousal response to the situation. However, this sensitivity to gaze direction, or arousal modulation, was not observed in the SCT group. Based on the results of this study, it can be suggested that the arousal system of children with SCT may respond differently than that of typically developing children. This could imply that children with SCT can depend less on their arousal system as a social ‘compass’ during social interactions, which could have consequences for how they respond and behave during these interactions. It is important to further explore arousal responses in social situations to gain a better understanding of how the arousal response relates to outcomes in children with SCT.

In addition to the SCT group as a whole, the role of SCT specific characteristics was also explored, including SCT karyotype (XXX, XXY, XYY), time of diagnosis, ascertainment bias, and research site. For none of the study outcomes (i.e., attention to the face, the eyes, and arousal sensitivity), an effect of these SCT characteristics was found. This suggests that the observed vulnerabilities in social orientation and arousal modulation may represent a rather 'stable' vulnerability associated with the genetic variation. It should be noted however, that results represent the *average* group of children with SCT and that there is always variability in outcomes, where some children are vulnerable, whereas other children will not differ from the control group.

Looking at the eyes and mouth of someone during social interactions may be impacted by the age of the child; younger children may focus more on the mouth during language learning, whereas this preferential looking might gradually shift to a preference to looking at the eyes. Also, sensitivity to difference in gaze direction might differ between younger and older children. For these reasons, the effect of age on group differences in looking times and sensitivity in arousal levels were explored further. No interaction effects were found for either time spent looking at the eyes, time spent looking at the mouth, or sensitivity in arousal modulation. Further examining this effect with correlations indicated that for time spent looking at the eyes, both the SCT and control group showed an increase with age. For time spent looking at the mouth the interaction effect did not indicate a different pattern between groups. However, correlations showed a significant decrease for time spent looking at the mouth in the control group, but not the SCT group. Lastly, for sensitivity in arousal modulation, there was no significant relation with age in either the control or SCT group. Taken together these results indicate that, although there might be differences between groups (i.e., children with SCT look less at the eyes) children with SCT do not appear to deviate from the control group more when they get older. This implies a persistent vulnerability across the entire 1–7-year age range, which suggests that it is possible that this vulnerability is anchored in the brain.

Relationships between looking behaviors and language outcomes, both concurrent and one year later were explored as well. Within the youngest age group (1-year-old children), significant correlations were found with both concurrent and future language outcomes. These results are in line with previous studies in typically developing children, and children with neurodevelopmental disorders, such as ASD (e.g., Habayeb et al., 2021; Lewkowicz & Hansen-Tift, 2012; Stagg et al., 2014; Tenenbaum et al., 2014; Tenenbaum et al., 2015; Young et al., 2009). The high correlations found in this age group illustrate that social orientation and language are intertwined at a very young age. It should be noted however, that no causal conclusions can be drawn from this; it remains unclear if more orientation to the mouth leads to better language abilities, or if children with better language abilities are more able to scan for socially relevant aspects, thus if better language abilities lead to more social orientation. With increasing age, typically developing children show a developmental change in orientation to the eyes versus the mouth (Frank et al., 2012). As a result, attention to specific areas of the face may contribute to language learning during specific developmental stages. Our findings fit with the proposition that with increasing age, attention to the mouth becomes less important for language learning, and that at a certain age, children may have passed this point (Tenenbaum et al., 2014).

When taking the results from the eye tracking and arousal together, the results of this study hint at a reduced ability to understanding and/or responding to social communicative demands in the environment. In other words, children with SCT might have a broader communication deficit. If children with SCT are less able to adapt to situational demands, this might explain why children with SCT also experience difficulties with language and other aspects of communication (Ross et al., 2008; Ross et al., 2009; St John et al., 2019; Urbanus, Swaab, Tartaglia, Boada, et al., 2021; Urbanus, Swaab, Tartaglia, Stumpel, et al., 2021; Zampini et al., 2020; Zampini et al., 2018), and why there are increased reports of social difficulties and social-emotional behavioral problems (Freilinger et al., 2018; Hong & Reiss, 2014; Urbanus et al., 2020; Visootsak & Graham, 2009). This study illustrates that nonverbal communication, that is needed to navigate social communicative interactions, consist of several important aspects, and that children with SCT experience difficulties with at least some of these aspects in areas of social attention and arousal responses.

This study comes with important clinical and scientific implications. Results of this study suggest that the presence of an extra X or Y chromosome may impact systems involved in social communication, not merely language systems. This is in line with neuroimaging studies, that demonstrate the impact of an extra sex chromosome on cortical regions that are part of the ‘social brain’ (e.g., Raznahan et al., 2016). It is recommended that future studies and clinicians take into account the broader domain of communication, in addition to structural language outcomes in children with SCT. This should be done from a young age, as both orientation difficulties and reduced arousal modulation were found irrespective of age. In addition, as language develops rapidly at a young age, language difficulties are already present in very young children with SCT, and language and social orientation are highly correlated in young children, results from this study point at an important window of opportunity to target social orientation and language in young children with SCT.

A relative strength of this study was that a large international sample of young children with SCT was included. Within this diverse group, no effect of recruitment site, time of diagnosis, or ascertainment bias was found, indicating that the included sample may be an adequate representation of the population of diagnosed children with SCT. It should be noted that although there were significant differences in IQ and SES between the control and SCT group, IQ and SES were not significantly correlated with our main parameters of interest. This in line with previous work (Van Rijn et al., 2018), illustrating that the use of eye tracking is a reliable measure to assess group differences regardless of level of functioning. In addition to strengths, some limitations of this study should also be noted. Although eye tracking allows for an ecological valid way to study looking behaviors, and we used a naturalistic situation, children might respond differently to watching a video as compared to a real life situation. Although we found reduced attention to the eyes in the SCT group while watching a video clip, we cannot conclude that these children also show reduced attention to the eyes in daily interactions. Also, both the effect of age and the effect of SCT karyotype were assessed separately. Due to the sample sizes, we were not able to look at age dependent effects within SCT karyotypes, which is an important direction for future studies. Sample sizes were smaller for our predictions between social orientation and language over time. Largely due to the world-wide COVID pandemic, we were unable to assess language one year after baseline assessment for some children. This resulted in small sample sizes in some of the age groups. Also, within this study, we only looked at the relationship between semantic language outcomes and social orientation, whereas other aspects of language, such as syntax or pragmatic language, might also be related to social orientation, in particular in older children (Çetinçelik et al., 2021). Lastly, results showed a diminished arousal modulation, and even though overall arousal level is relevant and interesting it does not inform us about type of emotions that are experienced.

To conclude, this study suggests that young children with SCT may have reduced orientation to social cues in response to social communication. In addition, the arousal system of children with SCT may be less sensitive to social cues. As social orienting abilities were related to longitudinal language abilities in the youngest group of children, this stresses the importance of targeting social orientation in early intervention programs.

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Supplementary Materials

Correlations between Parameters and Impact of SCT Characteristics on Outcomes

Table A. Correlations between IQ, SES, and global eye tracking parameters in the SCT group

	Screen	Face	Objects	ΔHR Direct Gaze (frontal)	ΔHR Indirect Gaze (side)
IQ	.09	-.19	.10	.11	.06
SES	-.12	-.08	.00	.04	.19

Table B. Impact of SCT characteristics on eye tracking outcomes: Time of diagnosis, ascertainment bias, and research site

		Time of Diagnosis[a]			**Ascertainment Bias[b]**				**Research Site[a]**		
		Prenatal	**Postnatal**	***p***	**A**	**B**	**C**	***p***	**USA**	**NL/BE**	***p***
Eye Tracking	*N*	48	26		33	23	18		38	36	
	Face	28.76	28.35	.881	28.38	29.68	27.68	.821	27.14	30.02	.262
		(1.57)	(2.18)		(11.02)	(11.10)	(8.76)		(1.79)	(1.74)	
	Eyes	10.04	12.53	.248	9.58	12.49	11.37	.432	9.32	12.44	.117
		(1.21)	(1.68)		(8.59)	(8.78)	(7.44)		(1.38)	(1.34)	
Arousal	*N*	39	26		31	21	13		34	31	
	Sensitivity	.341	1.39	.512	1.38	-1.41	2.80	.073	1.24	.23	.491
		(.95)	(1.19)		(6.08)	(4.89)	(4.87)		(.99)	(1.04)	

[a] Comparisons with (M)ANCOVA Scores represented Estimated Marginal Means (SE), corrected for age
[b] Comparisons with (M)ANOVA. Scores represent Means (SD). Ascertainment bias: A = Active prospective follow-up; B = Information seeking parents; C = Clinically referred cases